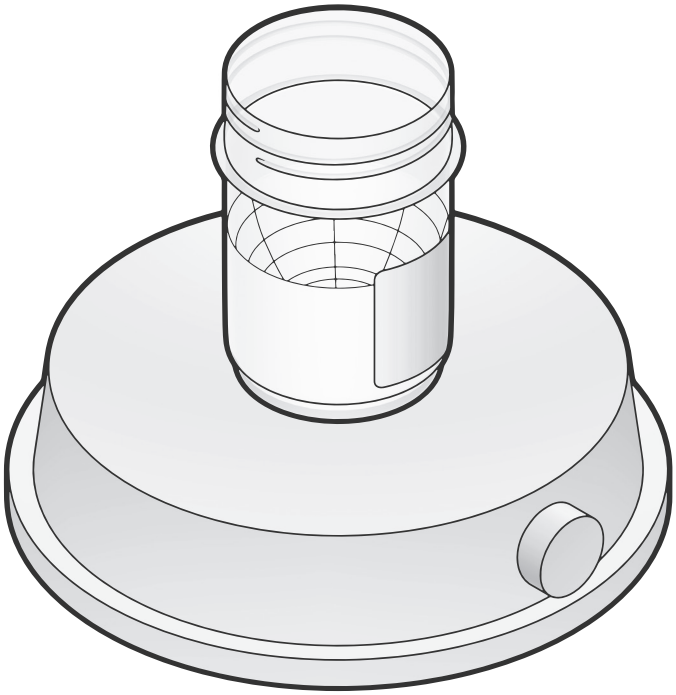


foodproof®

StarPrep® Two Kit

Spoilage Yeasts

Product Instructions

Documentation for the rapid extraction of
DNA for direct use in PCR

Product No.: KIT230177

foodproof®

StarPrep® Two Kit

Spoilage Yeasts

Store kit at 15 °C to 25 °C
For food testing purposes
FOR *IN VITRO* USE ONLY

Product No.: KIT230177
42 mL volume

Manual:
Revision A, December 2023

TABLE OF CONTENTS

1. OVERVIEW

The foodproof® StarPrep® Two Kit is designed for the rapid preparation of DNA from bacteria or yeast and mold for direct use in PCR. The extracted DNA can be used directly in any PCR application. The StarPrep Two Lysis Buffer eliminates the need for hazardous organic extractions or chaotropic agents. The entire DNA preparation can be performed in a single tube, minimizing handling steps and exposure to hazardous material. The reduced number of handling steps saves time. Transfer steps of DNA containing extracts are not necessary, thus cross-contamination risks are minimized.

1.1 General Information

Number of Reactions

The kit is designed for 96 reactions.

Storage Conditions

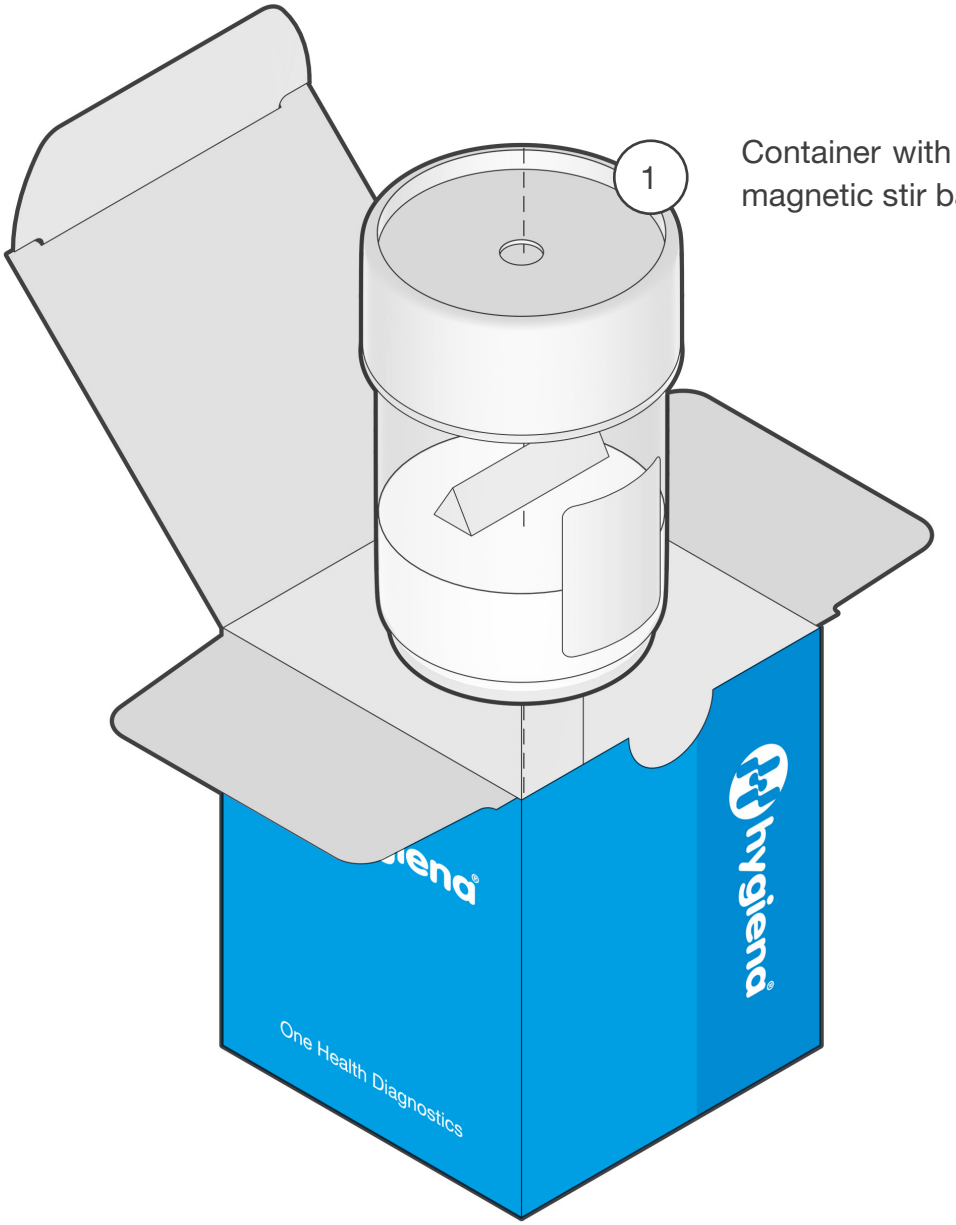
Store at 15 to 25 °C.
The components of the foodproof StarPrep Two Kit are guaranteed to be stable through the expiration date printed on the label.

1.2 Applicability

The lysis buffer is optimized for the preparation of various types of sample material, including enrichment cultures, direct samples and filtered samples. The sample volume varies depending on which matrix is being tested. For very cloudy supernatants, or samples containing inhibitors, a reduction of the sample volume may enhance the DNA isolation efficiency. The quality of the DNA obtained with the lysis buffer is suitable for any PCR application.

1.3 Kit Contents

A schematic representation of the foodproof StarPrep Two Kit with all its components.



1 Container with 42 mL lysis buffer and magnetic stir bar

2. INSTRUCTIONS

This section provides all information for a seamless DNA extraction from a variety of matrices.

2.1 Required Material

Most of the required equipment and reagents are available through Hygiena®. Please contact us for further information.

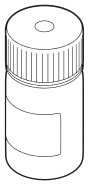
It is highly recommended only to use the materials described below to guarantee the robustness of the method.

Reagents

☐ **Reagent D**

Product No. KIT 2300 01/02/03

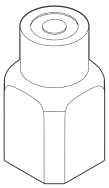
For Procedure B (2.3.2) to E (2.3.5)



☐ **Rinse Buffer**

Product No. KIT 2300 12

Only For Procedure E (2.3.5)



☐ **Reagent P**

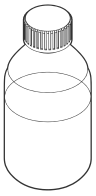
Product No. KIT 2300 07

Only For Procedure F (2.3.6)

☐ **Tris Buffer pH 8.0 1M**

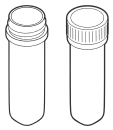
e.g., Trizma® hydrochloride solution

Only For Procedure F (2.3.6)



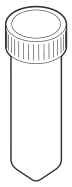
Consumables

☐ Sterile **2 mL reaction tubes with transparent screw caps**



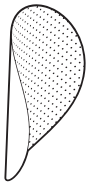
For Procedure D (2.3.4), E (2.3.5), and F (2.3.6)

☐ **5 mL tubes** (58 mm x 15 mm)



Only For Procedure E (2.3.5)

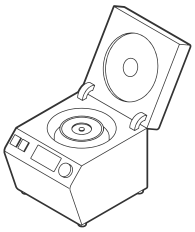
☐ **PES membrane filters, 1**
Pore size: 0.45 µm, diameter 47 mm; or
pore size: 0.22 µm, diameter: 47 mm



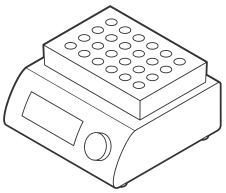
Only For Procedure E (2.3.5.)

Equipment

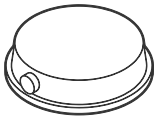
☐ Standard tabletop **microcentrifuge** capable of a 13,000 × g centrifugal force
e.g., Micro Star 21



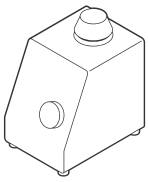
☐ **Heating unit** suitable for 1.5 mL tubes
e.g., AccuBlock™ - Labnet with heating block



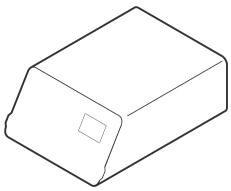
☐ **Magnetic stirrer**
e.g., Color squid IKAMAG® - IKA®-Werke



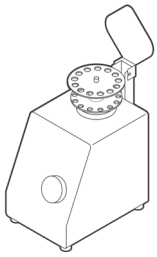
☐ **Vortex mixer**
e.g., Vortex-Genie® - Scientific Industries



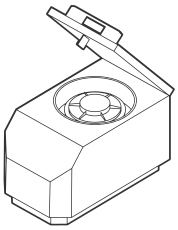
☐ **D-Light**
Product No.MCH2300 39
(Alternative: high-power blue LED incubation unit)
For Procedure B (2.3.2) to E (2.3.5)



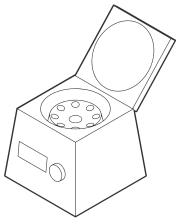
☐ Unit for **mechanical cell disruption** suitable for working with 1.5 mL reaction tubes
e.g., Mortexer™ - Benchmark Scientific
or Disruptor Genie® - Scientific Industries
For Procedure A (2.3.1), B (2.3.2), C (2.3.3), and G (2.3.7)



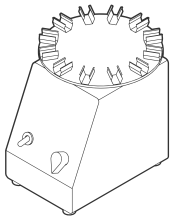
☐ Unit for **mechanical cell disruption** suitable for working with 2 mL reaction tubes
GeneReady - Hangzhou Lifereal Biotechnology
or BeadBug™ - Benchmark Scientific
For other devices, please inquire (see 2.5. Support).
For Procedure D (2.3.4) to F (2.3.6)



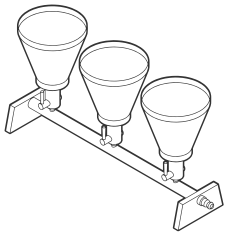
☐ **Centrifuge** suitable for 5 - 50 mL tubes and capable of a 3,500 × g centrifugal force
For Procedure E (2.3.5) and F (2.3.6)



☐ **Vortex mixer**
e.g., Vortex-Genie 2® - Scientific Industries with Large Ampule/Tube Attachment adapter or Horizontal 15 mL Tube Holder
Only For Procedure E (2.3.5)



☐ **Filtration unit** suitable for 47 mm filter membranes
Only For Procedure E (2.3.5)

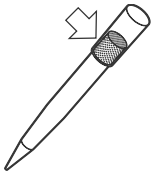


2.2 Precautions and Preparations

Follow all universal safety precautions governing work with biohazardous materials, e.g., wear lab coats and gloves at all times. Properly dispose of all contaminated materials, decontaminate work surfaces, and use a biosafety cabinet whenever aerosols might be generated.

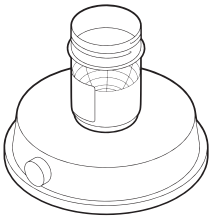
For more information, please refer to the appropriate material safety data sheet (MSDS). The MSDS is available online at www.hygiena.com/sds.

☐ Always use filter tips in order to avoid cross-contamination.

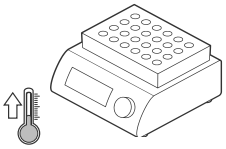


☐ Mix thoroughly while pipetting the buffer for sample preparation.

It is not recommended to use more than 96 reactions. The container must retain some of the reagent. Do not use anymore reagent once the minimum level mark on the container has been reached. The mark indicates the minimal allowed pipetting level while the stirrer is not in use.



☐ Set the heating unit to 95 to 100 °C.



☐ Prepare Reagent P before using the first time.

Dissolve Reagent P in 5 mL double-distilled water, aliquot solution.

Store at –15 to –25 °C, stable for 12 months.

2.3 Workflows

The following procedures describe the DNA isolation from different types of samples and beverages. Depending on your sample, please choose the appropriate method from the table below.

EXTRACTION PROCEDURE A is a quick protocol without live / dead cell differentiation.

EXTRACTION PROCEDURE B allows a high sample volume for a higher sensitivity.

EXTRACTION PROCEDURE C is a quick protocol including live / dead cell differentiation.

WORKFLOWS

EXTRACTION PROCEDURE D is suitable for direct sampling without enrichment.
EXTRACTION PROCEDURE E is for direct analysis of filtered samples without enrichment.
EXTRACTION PROCEDURE F is intended for samples with high yeast content.
EXTRACTION PROCEDURE G is intended for identification of colonies.

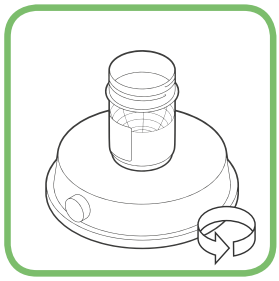
Sample Type	Method	Analysis	Procedure
non-alcoholic beverages, alcoholic beverages, alcoholic mixed drinks, syrup	liquid enrichment	absence/ presence/ *quick protocol without Reagent D*	A
		absence/ presence/ *improved sensitivity*	B
unfiltered beer, pasteurized beverages with high yeast content	liquid enrichment	absence/ presence	C
non-alcoholic beverages, alcoholic beverages, alcoholic mixed drinks, syrup	direct analysis	absence/ presence	D
		quantification	D
filtered beer, clear shandies, lemonade, flavored water	filtration	absence/ presence	E
		quantification	E
dry yeast, yeast slurry, yeast enrichment culture	direct analysis or liquid enrichment	absence/ presence	F
all sample types	nutrient agar	colony confirmation	G

Note: Samples containing plant material or extracts from plants or fruits will inhibit the PCR reaction. It is currently not recommended to perform direct analysis on such samples and enrichment cultures should be prepared with adequate dilution in a suitable broth (e.g., 1:10 dilution in YM broth).

2.3.1 EXTRACTION PROCEDURE A

This quick protocol is intended for detection of spoilage yeast in enrichment cultures.

Note: This protocol may not be suitable for the detection of viable Saccharomyces spp. or other yeast species in samples that contain high levels of dead yeast cells, e.g., pasteurized unfiltered beer. Use procedure C for such samples.



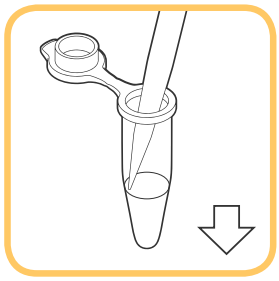
1. PREPARE LYSIS BUFFER

Place closed lysis buffer container on the magnetic stirrer.

Continuously mix lysis buffer at 400 rpm on the magnetic stirrer to keep solution homogeneous.

Open the lysis buffer container.

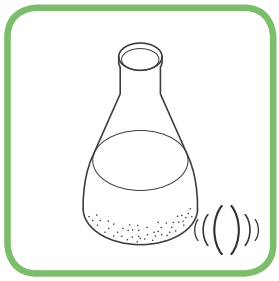
Note: Hold the container while switching on the magnetic stirrer and during pipetting.



2. ADD LYSIS BUFFER

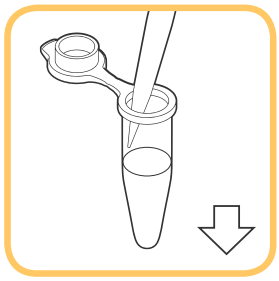
Transfer **300 µL** lysis buffer to a 1.5 mL reaction tube.

Note: Pipet carefully and vertically along the lysis buffer container wall, approximately 0.5 cm above the bottom. Use a 1,000 µL filter tip to transfer lysis buffer to the reaction tube. Depending on the mechanical disruption unit it is also possible to use 2 mL reaction tubes.



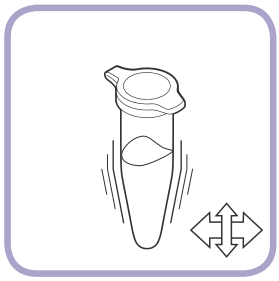
3. SHAKE SAMPLE

Shake enrichment culture (or beverage sample) gently and let the suspension settle for 5 to 10 min.



4. ADD SAMPLE

Transfer **25 µL** sample (enrichment culture supernatant) to the reaction tube containing the lysis buffer.

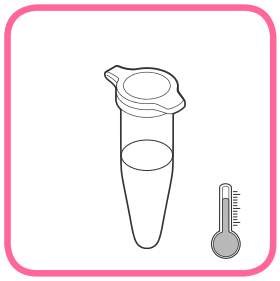


5. MECHANICAL DISRUPTION

Place tube in a cell disruption unit and perform disruption:

Mortexer or Disruptor Genie: 8 min at maximum speed.

Note: The efficiency of disruption depends on the mechanical cell disruption unit.



6. INCUBATE

5 min at 95 to 100 °C in a heating unit.

Carefully remove the reaction tube from the heating unit and allow the tube to sit for **1 min at 15 to 25 °C**.

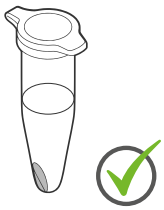
7. MIX

Vortex for 2 sec.

8. CENTRIFUGE

1 min at 13,000 x g.

Note: If necessary, centrifugation forces should be calculated according to the centrifuge users manual.



SUPERNATANT FOR DETECTION

Use extract for the foodproof PCR kits.

Strictly avoid transferring fractions of the sediment to the PCR reaction, because this might cause PCR inhibition.

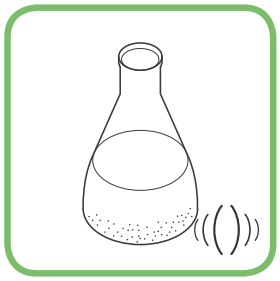
For later analysis, store DNA at -15 to -25 °C.

After thawing, mix briefly by vortexing and centrifuge at 13,000 × g for 1 min.

2.3.2 EXTRACTION PROCEDURE B

This protocol is intended for detection of low levels of spoilage yeast in enrichment cultures. It is especially useful for detecting slow growing species such as *Dekkera* spp.

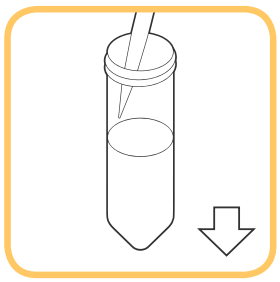
Note: This protocol may not be suitable for the detection of viable Saccharomyces spp. or other yeast species in samples that contain high levels of dead yeast cells, e.g., pasteurized unfiltered beer. For such samples, please use procedure C.



1. SHAKE SAMPLE

Shake enrichment culture (or beverage sample) gently and let the suspension settle for 5 to 10 min.

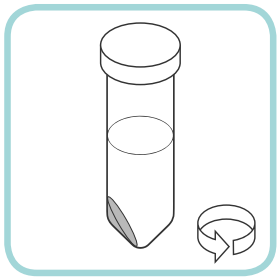
OPTIONAL PRE-CONCENTRATION



A. ADD SAMPLE

Transfer **up to 30 mL** sample (supernatant) to a 50 mL tube.

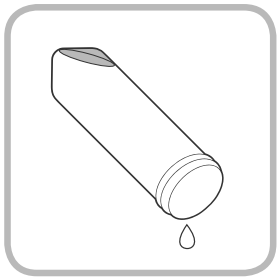
Note: Pre-concentration significantly improves sensitivtiy. Not recommended for very cloudy samples.



B. CENTRIFUGE

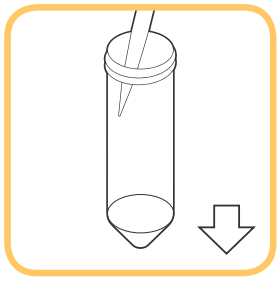
20 min at 1,800 x g.

Note: If necessary, centrifugation forces should be calculated according to the centrifuge users manual.



C. DECANT SUPERNATANT

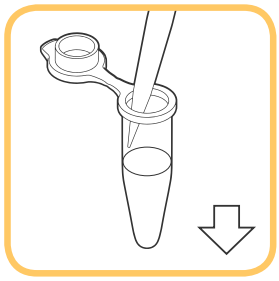
Decant supernatant completely.



D. RESUSPEND

Transfer **1 mL** Rinse Buffer and resuspend pellet by pipetting up and down 5 to 10 times or brief vortexing.

Note: Alternatively, resuspend with Buffered Peptone Water.



2. ADD SAMPLE

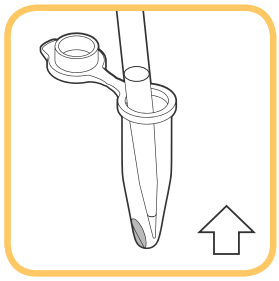
Transfer **up to 1,000 µL** sample from step 1 or step D to a 1.5 mL reaction tube.

Note: For samples known to inhibit PCR, reduce the volume, e.g., to 100 µL.

3. CENTRIFUGE

5 min at 8,000 x g.

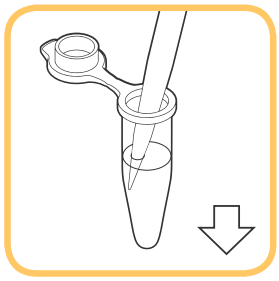
Note: If necessary, centrifugation forces should be calculated according to the centrifuge users manual.



4. REMOVE SUPERNATANT

Discard liquid with a pipette immediately after centrifugation and inactivate appropriately.

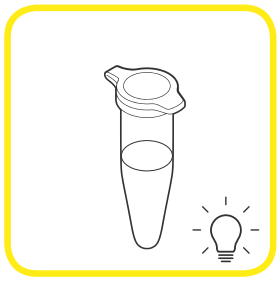
Note: Take care that the tip of the pipette is on the opposite side of the pellet during pipetting.



5. ADD REAGENT D

Transfer **300 µL** Reagent D and mix by pipetting up and down 5 to 10 times or brief vortexing.

Note: Proceed immediately with the following steps of the protocol.
Avoid extended exposure to light.
For optimal efficiency, the mixing has to be complete.



6. D-LIGHT TREATMENT

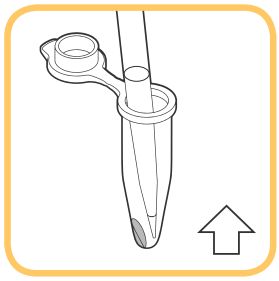
Incubate for **10 min at room temperature** in the D-Light **in the dark.**

Incubate for **5 min at room temperature** in the D-Light **with light exposure.**

7. CENTRIFUGE

5 min at 8,000 x g.

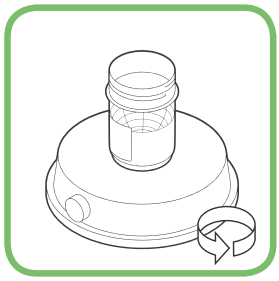
Note: If necessary, centrifugation forces should be calculated according to the centrifuge users manual.



8. REMOVE SUPERNATANT

Discard liquid with a pipette immediately after centrifugation and inactivate appropriately.

Note: Take care that the tip of the pipette is on the opposite side of the pellet during pipetting.



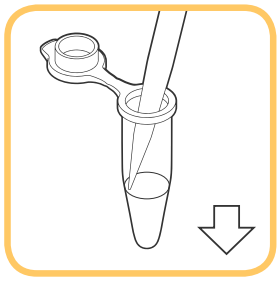
9. PREPARE LYSIS BUFFER

Place closed lysis buffer container on the magnetic stirrer.

Continuously mix lysis buffer at 400 rpm on the magnetic stirrer to keep solution homogeneous.

Open the lysis buffer container.

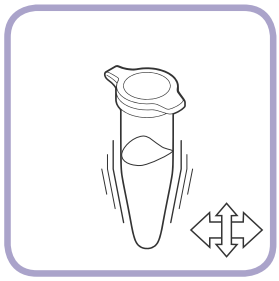
Note: Hold the container while switching on the magnetic stirrer and during pipetting.



10. ADD LYSIS BUFFER

Transfer **300 µL** lysis buffer to a 1.5 mL reaction tube.

Note: Pipet carefully and vertically along the lysis buffer container wall, approximately 0.5 cm above the bottom.
Use a 1,000 µL filter tip to transfer lysis buffer to the sample.

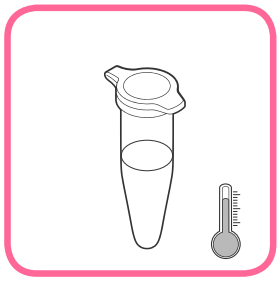


11. MECHANICAL DISRUPTION

Place tube in a cell disruption unit and perform disruption:

Mortexer or Disruptor Genie: 8 min at maximum speed.

Note: The efficiency of disruption depends on the mechanical cell disruption unit.



12. INCUBATE

5 min at 95 to 100 °C in a heating unit.

Carefully remove the reaction tube from the heating unit and allow the tube to sit for **1 min at 15 to 25 °C**.

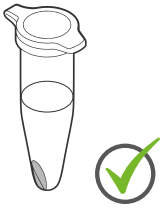
13. MIX

Vortex for 2 sec.

14. CENTRIFUGE

1 min at 13,000 x g.

Note: If necessary, centrifugation forces should be calculated according to the centrifuge users manual.



SUPERNATANT FOR DETECTION

Use extract for the foodproof PCR kits.

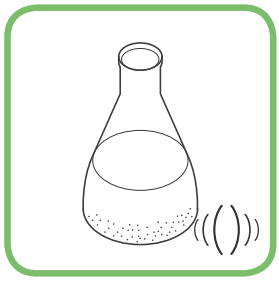
Strictly avoid transferring fractions of the sediment to the PCR reaction, because this might cause PCR inhibition.

For later analysis, store DNA at -15 to -25 °C.

After thawing, mix briefly by vortexing and centrifuge at 13,000 × g for 1 min.

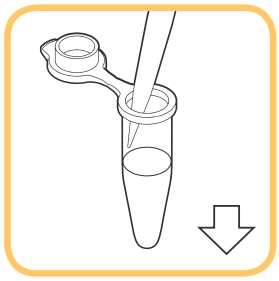
2.3.3 EXTRACTION PROCEDURE C

This protocol is intended for detection of spoilage by yeast strains used in the brewing or fermentation process and/or closely related species, e.g., *Saccharomyces* spp. It is particularly useful for detection of viable *Saccharomyces* spp. in pasteurized unfiltered beer.



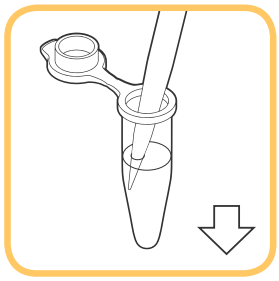
1. SHAKE SAMPLE

Shake enrichment culture gently and let the suspension settle for 5 to 10 min.



2. ADD SAMPLE

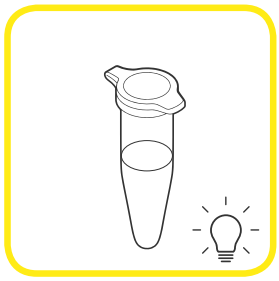
Transfer **25 µL** sample (enrichment culture supernatant) to a transparent 1.5 mL reaction tube.



3. ADD REAGENT D

Transfer **300 µL** Reagent D and mix by pipetting up and down or by briefly vortexing.

Note: Proceed immediately with the following steps of the protocol.
Avoid extended exposure to light.
For optimal efficiency, the mixing has to be complete.



4. D-LIGHT TREATMENT

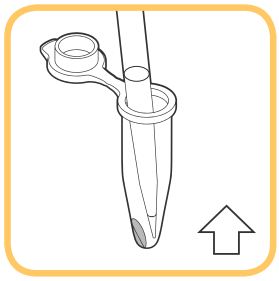
Incubate for **10 min at room temperature** in the D-Light **in the dark.**

Incubate for **5 min at room temperature** in the D-Light **with light exposure.**

5. CENTRIFUGE

5 min at 8,000 x g.

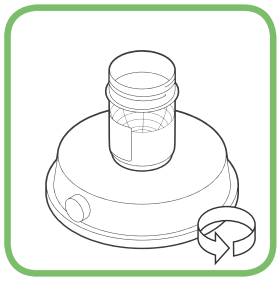
Note: If necessary, centrifugation forces should be calculated according to the centrifuge users manual.



6. REMOVE SUPERNATANT

Discard liquid with a pipette immediately after centrifugation and inactivate appropriately.

Note: Take care that the tip of the pipette is on the opposite side of the pellet during pipetting.



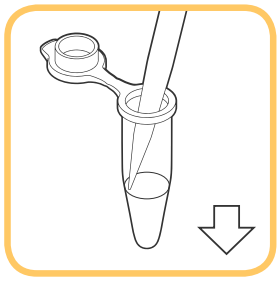
7. PREPARE LYSIS BUFFER

Place closed lysis buffer container on the magnetic stirrer.

Continuously mix lysis buffer at 400 rpm on the magnetic stirrer to keep solution homogeneous.

Open the lysis buffer container.

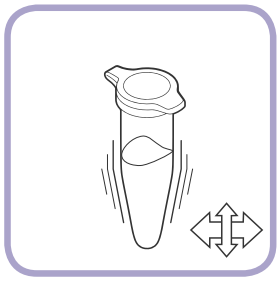
Note: Hold the container while switching on the magnetic stirrer and during pipetting.



8 ADD LYSIS BUFFER

Transfer **300 µL** lysis buffer to a 1.5 mL reaction tube.

Note: Pipet carefully and vertically along the lysis buffer container wall, approximately 0.5 cm above the bottom.
Use a 1,000 µL filter tip to transfer lysis buffer to the sample.

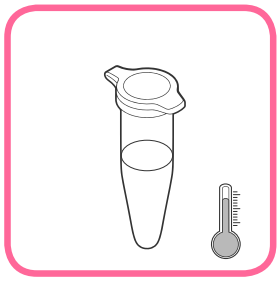


9. MECHANICAL DISRUPTION

Place tube in a cell disruption unit and perform disruption:

Mortexer or Disruptor Genie: 8 min at maximum speed.

Note: The efficiency of disruption depends on the mechanical cell disruption unit.



10. INCUBATE

5 min at 95 to 100 °C in a heating unit.

Carefully remove the reaction tube from the heating unit and allow the tube to sit for **1 min at 15 to 25 °C**.

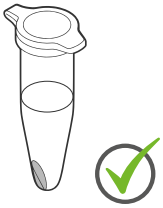
11. MIX

Vortex for 2 sec.

12. CENTRIFUGE

1 min at 13,000 x g.

Note: If necessary, centrifugation forces should be calculated according to the centrifuge users manual.



SUPERNATANT FOR DETECTION

Use extract for the foodproof PCR kits.

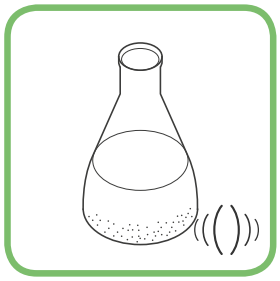
Strictly avoid transferring fractions of the sediment to the PCR reaction, because this might cause PCR inhibition.

For later analysis, store DNA at -15 to -25 °C.

After thawing, mix briefly by vortexing and centrifuge at 13,000 × g for 1 min.

2.3.4 EXTRACTION PROCEDURE D

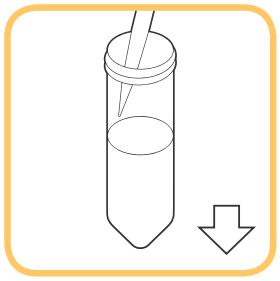
This protocol is intended for detection of spoilage yeasts directly from beverage samples without enrichment. This protocol is also suitable for quantification of spoilage yeasts.



1. SHAKE SAMPLE

Shake enrichment culture (or beverage sample) gently and let the suspension settle for 5 to 10 min.

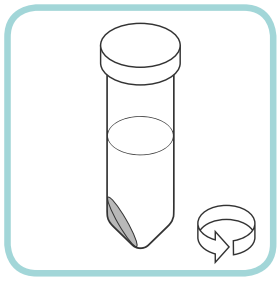
OPTIONAL PRE-CONCENTRATION



A. ADD SAMPLE

Transfer **up to 30 mL** sample (supernatant) to a 50 mL tube.

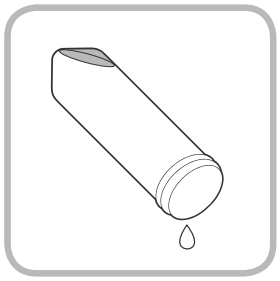
Note: Pre-concentration significantly improves sensitivtiy. Not recommended for very cloudy samples.



B. CENTRIFUGE

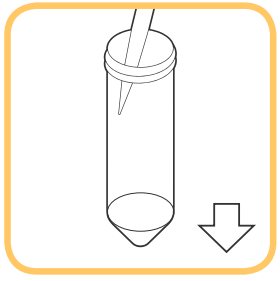
20 min at 1,800 x g.

Note: If necessary, centrifugation forces should be calculated according to the centrifuge users manual.



C. DECANT SUPERNATANT

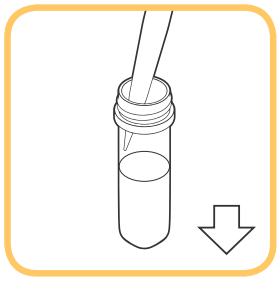
Decant supernatant completely.



D. RESUSPEND

Transfer **1 mL** Rinse Buffer and resuspend pellet by pipetting up and down 5 to 10 times or brief vortexing.

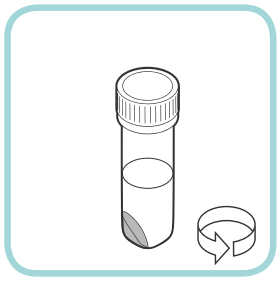
Note: Alternatively, resuspend with Buffered Peptone Water.



2. ADD SAMPLE

Transfer **up to 1,000 µL** sample from step 1 or step D to a transparent 2 mL screw cap tube.

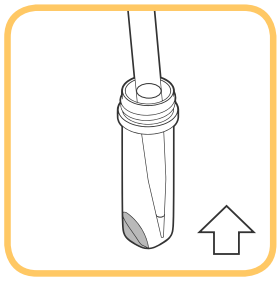
Note: For samples known to inhibit PCR, reduce the volume, e.g., to 100 µL.



3. CENTRIFUGE

5 min at 8,000 x g.

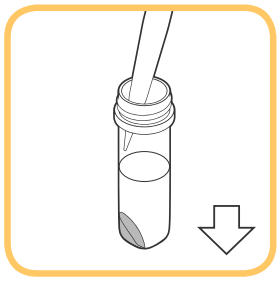
Note: If necessary, centrifugation forces should be calculated according to the centrifuge users manual.



4. REMOVE SUPERNATANT

Discard liquid with a pipette immediately after centrifugation and inactivate appropriately.

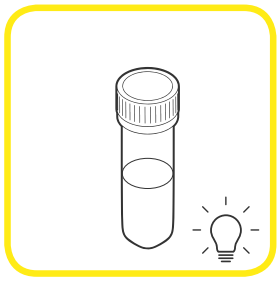
Note: Take care that the tip of the pipette is on the opposite side of the pellet during pipetting.



5. ADD REAGENT D

Transfer **300 µL** Reagent D and mix by pipetting up and down 5 to 10 times or by briefly vortexing.

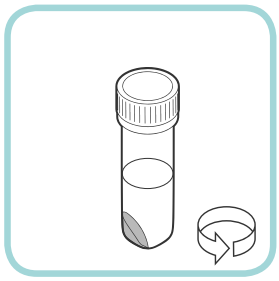
Note: Proceed immediately with the following steps of the protocol.
Avoid extended exposure to light.
For optimal efficiency, the mixing has to be complete.



6. D-LIGHT TREATMENT

Incubate for **10 min at room temperature** in the D-Light **in the dark.**

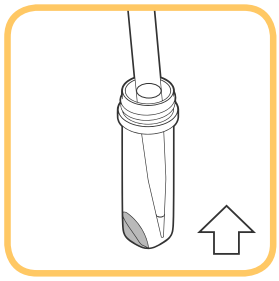
Incubate for **5 min at room temperature** in the D-Light **with light exposure.**



7. CENTRIFUGE

5 min at 8,000 x g.

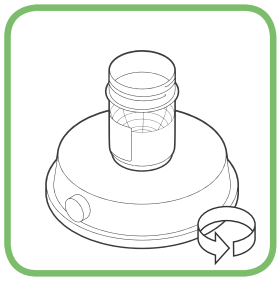
Note: If necessary, centrifugation forces should be calculated according to the centrifuge users manual.



8. REMOVE SUPERNATANT

Discard liquid with a pipette immediately after centrifugation and inactivate appropriately.

Note: Take care that the tip of the pipette is on the opposite side of the pellet during pipetting.



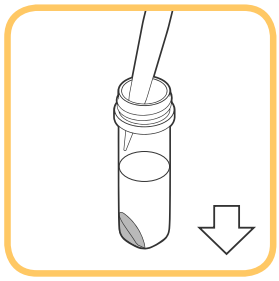
9. PREPARE LYSIS BUFFER

Place closed lysis buffer container on the magnetic stirrer.

Continuously mix lysis buffer at 400 rpm on the magnetic stirrer to keep solution homogeneous.

Open the lysis buffer container.

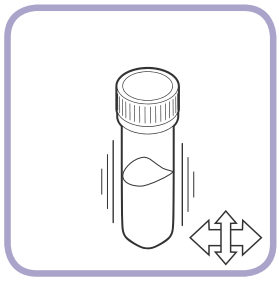
Note: Hold the container while switching on the magnetic stirrer and during pipetting.



10. ADD LYSIS BUFFER

Transfer **300 µL** lysis buffer to a 2 mL screw cap tube.

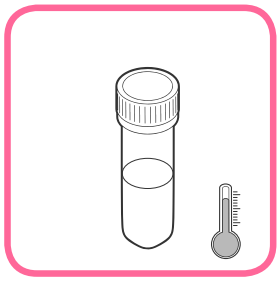
Note: Pipet carefully and vertically along the lysis buffer container wall, approximately 0.5 cm above the bottom.
Use a 1,000 µL filter tip to transfer lysis buffer to the sample.



11. MECHANICAL DISRUPTION

Place tube in a cell disruption unit and perform disruption:

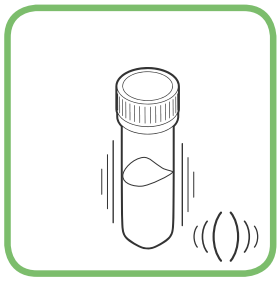
GeneReady - 4 min at 6.5 m/s or
BeadBug - 2 min at 4,000 rpm



12. INCUBATE

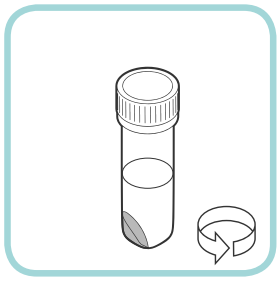
5 min at 95 to 100 °C in a heating unit.

Carefully remove the reaction tube from the heating unit and allow the tube to sit for **1 min at 15 to 25 °C**.



13. MIX

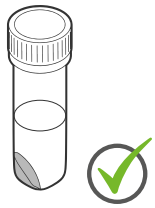
Vortex for 2 sec.



14. CENTRIFUGE

1 min at 13,000 x g.

Note: If necessary, centrifugation forces should be calculated according to the centrifuge users manual.



SUPERNATANT FOR DETECTION

Use extract for the foodproof® **PCR kits.**

Strictly avoid transferring fractions of the sediment to the PCR reaction, because this might cause PCR inhibition.

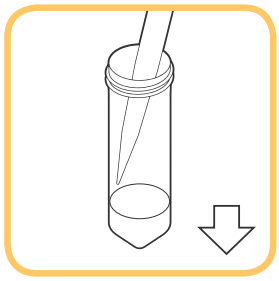
For later analysis, store DNA at -15 to -25 °C.

After thawing, mix briefly by vortexing and centrifuge at 13,000 × g for 1 min.

2.3.5 EXTRACTION PROCEDURE E

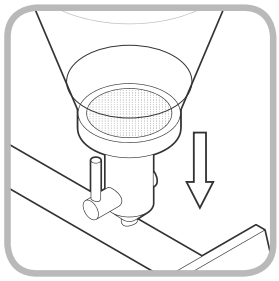
This protocol is intended for detection of spoilage yeast in beverage samples using 47 mm filter disks.

Note: To generate valid results, strictly adhere to the protocol below and only use the filter material, tubes and Rinse Buffer specified by Hygiena.



1. ADD RINSE BUFFER

Transfer **1,000 µL** Rinse Buffer to a 5 mL tube.

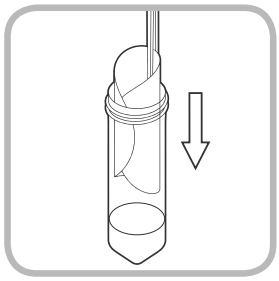


2. FILTER SAMPLES

Use a PES membrane to filter samples in a filtration unit.

Write down the filtration volume.

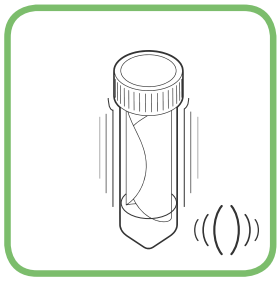
Note: Conduct filtration according to your laboratory's specifications. Only use PES filters.



3. FILTER PAPER TO RINSE BUFFER

Transfer the filter paper to the 5 mL tube containing the Rinse Buffer with the top side (filter cake) facing inwards**.**

Note: Pick up the filter disk at opposing sides using two sterile forceps. Gently roll the membrane into the shape of a cylinder until it fits into the 5 mL tube. Be careful not to crumple the filter disk or touch its upper side!

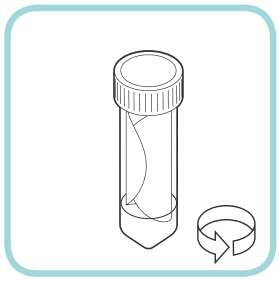


4. VORTEX

Insert tubes into "Large Ampule/Tube Attachment" or "Horizontal 15 mL Tube Holder" on a Vortex Genie 2.

Vortex for **15 sec at full speed**,
then vertically rotate the tubes 180° and **repeat** the process.

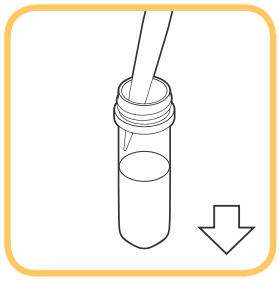
Note: The tubes should be balanced. When processing an odd number of samples, use a 5 mL tube filled with 1 mL distilled water as counterweight.



5. CENTRIFUGE

1 min at 1,000 x g.

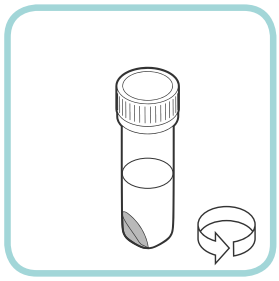
Note: Use a centrifuge suitable for 5 – 15 mL tubes. If necessary, centrifugation forces should be calculated according to the centrifuge users manual.



6. ADD SAMPLE

Transfer **700 µL** sample (from 5mL tube) to a transparent 2 mL screw cap tube.

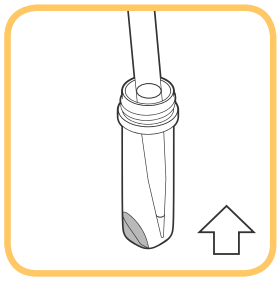
Note: In case recovery of 700 µL cannot be achieved, write down the approx. recovered volume and take this into account when calculating the yeast cell concentration.



7. CENTRIFUGE

5 min at 8,000 x g.

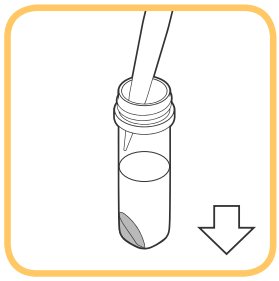
Note: If necessary, centrifugation forces should be calculated according to the centrifuge users manual.



8. REMOVE SUPERNATANT

Discard liquid with a pipette immediately after centrifugation and inactivate appropriately.

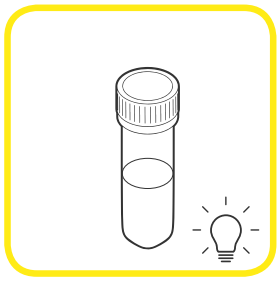
Note: Take care that the tip of the pipette is on the opposite side of the pellet during pipetting.



9. ADD REAGENT D

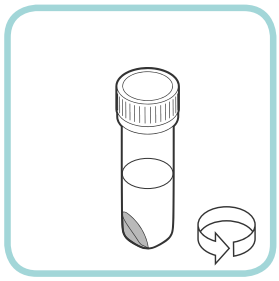
Transfer **300 µL** Reagent D and resuspend the pellet by pipetting gently up and down 5 to 10 times.

Note: Proceed immediately with the following steps of the protocol.
Avoid extended exposure to light.
For optimal efficiency, the pellet has to be completely resuspended.



10. D-LIGHT TREATMENT

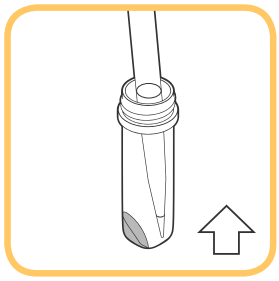
Incubate for **10 min at room temperature** in the D-Light **in the dark,**
Incubate for **5 min at room temperature** in the D-Light **with light exposure.**



11. CENTRIFUGE

5 min at 8,000 x g.

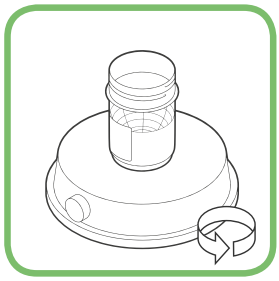
Note: If necessary, centrifugation forces should be calculated according to the centrifuge users manual.



12. REMOVE SUPERNATANT

Completely discard liquid with a pipette immediately after centrifugation and inactivate appropriately.

Note: Take care that the tip of the pipette is on the opposite side of the pellet during pipetting.



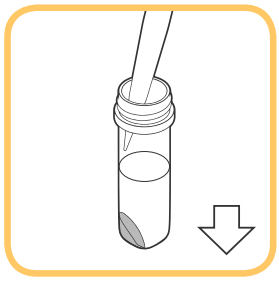
13. PREPARE LYSIS BUFFER

Place closed lysis buffer container on the magnetic stirrer.

Continuously mix lysis buffer at 400 rpm on the magnetic stirrer to keep solution homogeneous.

Open the lysis buffer container.

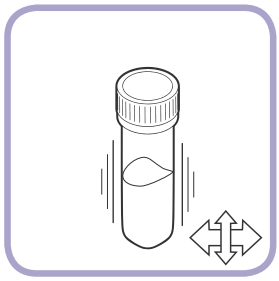
Note: Hold the container while switching on the magnetic stirrer and during pipetting.



14. ADD LYSIS BUFFER

Transfer **300 µL** lysis buffer to the tube with the sample pellet.

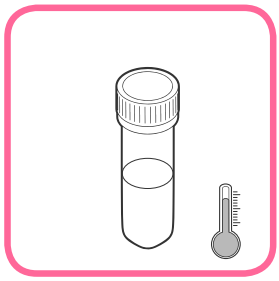
Note: Pipet carefully and vertically along the lysis buffer container wall, approximately 0.5 cm above the bottom.
Use a 1,000 µL filter tip to transfer lysis buffer to the sample.



15. MECHANICAL DISRUPTION

Place tube in a cell disruption unit and perform disruption:

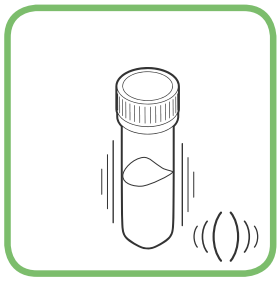
GeneReady - 4 min at 6.5 m/s or
BeadBug - 2 min at 4,000 rpm



16. INCUBATE

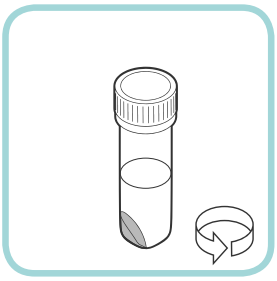
5 min at 95 to 100 °C in a heating unit.

Carefully remove the reaction tube from the heating unit and allow the tube to sit for **1 min at 15 to 25 °C**.



17. MIX

Vortex for 2 sec.



18. CENTRIFUGE

1 min at 13,000 x g.

Note: If necessary, centrifugation forces should be calculated according to the manual of the used centrifuge.

SUPERNATANT FOR DETECTION

Use extract for the foodproof PCR kits.

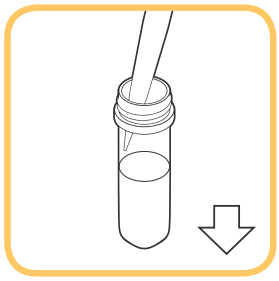
Strictly avoid transferring fractions of the sediment to the PCR reaction, because this might cause PCR inhibition.

For later analysis, store DNA at -15 to -25 °C.

After thawing, mix briefly by vortexing and centrifuge at 13,000 × g for 1 min.

2.3.6 EXTRACTION PROCEDURE F

This protocol is intended for detection of spoilage yeasts from pure yeast or and other samples with high yeast content. This includes commercial dry yeast and pitching yeast slurries. This protocol is also suitable for detection of spoilage yeasts in enrichment cultures of brewers yeast.

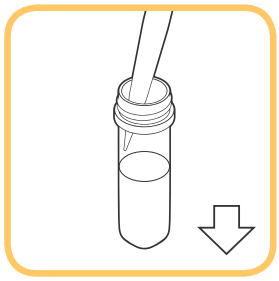


1A. PREPARE SAMPLE: DRY YEAST

Weigh up to 200 mg (50 mg recommended) sample in a 2 mL screw cap tube.

Add 1 mL of 100 mM Tris-buffer (pH 8.0).

Completely resuspend the dry yeast by vortexing.

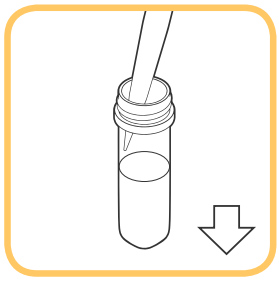


-or- 1B. PREPARE SAMPLE: YEAST SLURRY

Add **500 µL - 1,000 µL** slurry corresponding to approx. 10^9 yeast cells to a 2 mL screw cap tube.

Add **100 µL** of 1 M Tris-buffer (pH 8.0).

Briefly vortex.

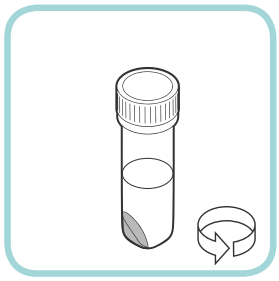


-or- 1C. PREPARE SAMPLE: ENRICHMENT CULTURE OF YEAST

Transfer up to **50 mL** (equivalent of approx. 10^9 yeast cells) enrichment culture to a centrifuge tube with a conical bottom.

Centrifuge at **3,500 x g for 15 min** and discard the supernatant.

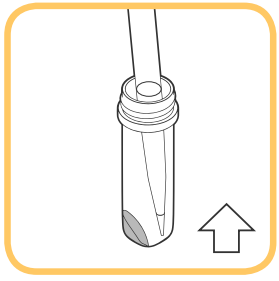
Resuspend the pellet in **1 mL** of 100 mM Tris-buffer (pH 8.0) and transfer up to 1.5 mL to a 2 mL screw cap tube.



2. CENTRIFUGE

5 min at 8,000 x g.

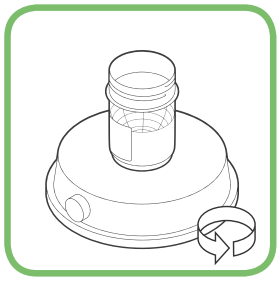
Note: If necessary, centrifugation forces should be calculated according to the centrifuge users manual.



3. REMOVE SUPERNATANT

Discard liquid with a pipette immediately after centrifugation and inactivate appropriately.

Note: Take care that the tip of the pipette is on the opposite side of the pellet during pipetting.



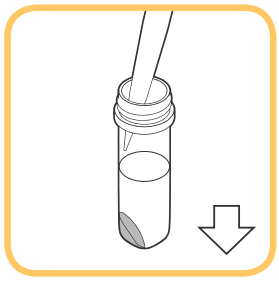
4. PREPARE LYSIS BUFFER

Place closed lysis buffer container on the magnetic stirrer.

Continuously mix lysis buffer at 400 rpm on the magnetic stirrer to keep solution homogeneous.

Open the lysis buffer container.

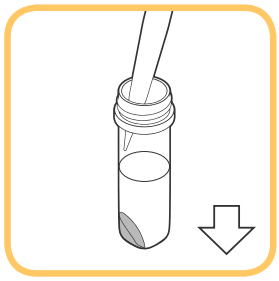
Note: Hold the container while switching on the magnetic stirrer and during pipetting.



5. ADD LYSIS BUFFER

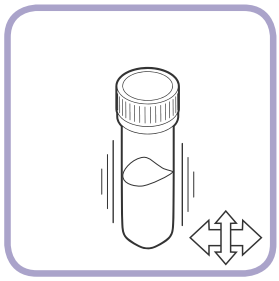
Transfer **300 µL** lysis buffer to a 2 mL screw cap tube.

Note: Pipet carefully and vertically along the lysis buffer container wall, approximately 0.5 cm above the bottom.
Use a 1,000 µL filter tip to transfer lysis buffer to the sample.



6. ADD REAGENT P

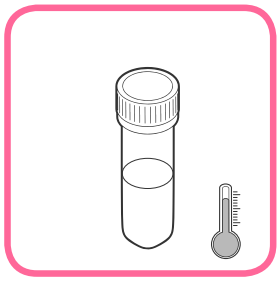
Transfer **50 µL** Reagent P.



7. MECHANICAL DISRUPTION

Place tube in a cell disruption unit and perform disruption:

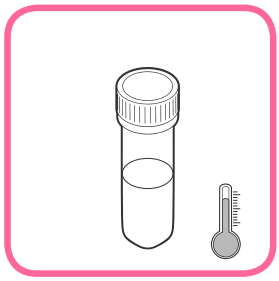
GeneReady - 4 min at 6.5 m/s or
BeadBug - 4 min at 4,000 rpm



8. INCUBATE I

60 min at 60 °C in a heating unit.

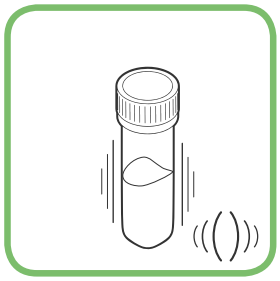
Note: Agitation at 500 rpm during the incubation period improves DNA extraction, but is not mandatory.



9. INCUBATE II

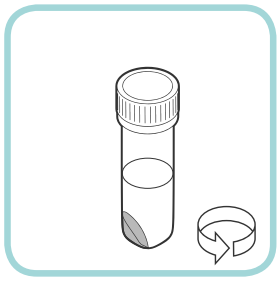
15 min at 95 to 100 °C in a heating unit.

Carefully remove the reaction tube from the heating unit and allow the tube to sit for **1 min at 15 to 25 °C**.



10. MIX

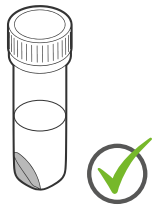
Vortex for 2 sec.



11. CENTRIFUGE

1 min at 13,000 x g.

Note: If necessary, centrifugation forces should be calculated according to the manual of the used centrifuge.



SUPERNATANT FOR DETECTION

Use 5 µL extract for the foodproof PCR kits.

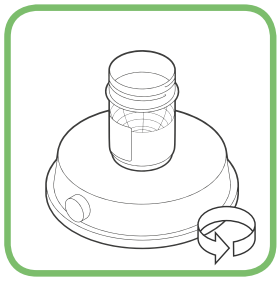
PCR inhibition may be observed when using more than 5 µL supernatant. Strictly avoid transferring fractions of the sediment to the PCR reaction, because this might cause PCR inhibition.

For later analysis, store DNA at -15 to -25 °C.

After thawing, mix briefly by vortexing and centrifuge at 13,000 × g for 1 min.

2.3.7 EXTRACTION PROCEDURE G

The following protocol describes the DNA isolation from colonies grown on agar plates.



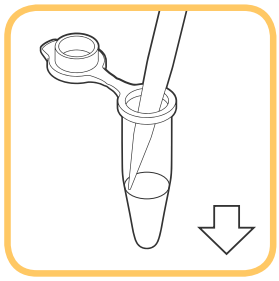
1. PREPARE LYSIS BUFFER

Place closed lysis buffer container on the magnetic stirrer.

Continuously mix lysis buffer at 400 rpm on the magnetic stirrer to keep solution homogeneous.

Open the lysis buffer container.

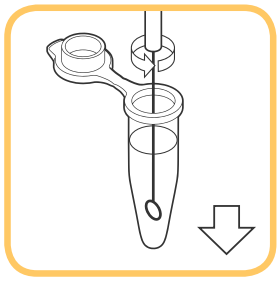
Note: Hold the container while switching on the magnetic stirrer and during pipetting.



2. ADD LYSIS BUFFER

Transfer **300 µL** lysis buffer to a 1.5 mL reaction tube.

Note: Pipet carefully and vertically along the lysis buffer container wall, approximately 0.5 cm above the bottom. Use a 1,000 µL filter tip to transfer lysis buffer to the sample. Depending on the mechanical disruption unit it is also possible to use a 2 mL reaction tube.

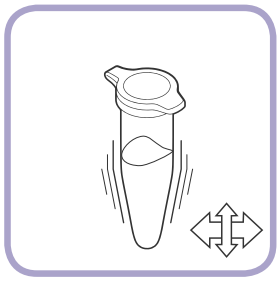


3. ADD PICKED COLONIES

Transfer a small part of the colony with a suitable tool (e.g., inoculating needle) to the reaction tube containing the lysis buffer.

4. MIX

Vortex for 2 sec.

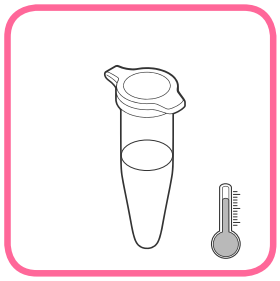


5. MECHANICAL DISRUPTION

Place tube in a cell disruption unit and perform disruption:

Mortexer or Disruptor Genie: 8 min at maximum speed.

Note: The efficiency of disruption depends on the mechanical cell disruption unit.



6. INCUBATE

5 min at 95 to 100 °C in a heating unit.

Carefully remove the reaction tube from the heating unit and allow the tube to sit for **1 min at 15 to 25 °C**.

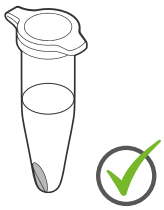
7. MIX

Vortex for 2 sec.

8. CENTRIFUGE

5 min at 13,000 x g.

Note: If necessary, centrifugation forces should be calculated according to the centrifuge users manual.



SUPERNATANT FOR DETECTION

Use extract for the foodproof PCR kits.

Strictly avoid transferring fractions of the sediment to the PCR reaction, because this might cause PCR inhibition.

For later analysis, store DNA at -15 to -25 °C.

After thawing, mix briefly by vortexing and centrifuge at 13,000 × g for 1 min.

2.4 Troubleshooting

Problem	Possible Cause	Recommendation
Extract inhibits PCR	Enrichment culture or sample contains too many PCR inhibitors.	Perform a subcultivation, e.g., 1:10 dilution in fresh enrichment broth. Repeat DNA extraction with a reduced sample volume.
	DNA extract contains too many PCR inhibitors.	Dilute DNA extract, e.g., 1:10, or reduce the amount of extracted DNA, e.g., for LyoKits 5 µL instead of 25 µL.
	Some of the centrifugation pellet transferred over to the PCR.	Always centrifuge the DNA sample before performing PCR. Use the top of the supernatant as a PCR template. Do not allow the filter tip to have contact with the pellet.
	Supernatants are not completely removed.	Remove supernatants completely (e.g., after Reagent D treatment).
Low DNA yield	Improper storage of kit components.	Store kit reagents at 15 to 25 °C.
	Enrichment culture contains substances that reduce the DNA extraction efficiency.	Perform a subcultivation or dilution, e.g., 1:10 in fresh enrichment broth.
	Sample contains substances that reduce the DNA extraction efficiency.	Reduce the sample volume.
	Not enough target organisms in enrichment culture.	Prolong the incubation phase.
	Pellet resuspension incomplete.	Improve resuspension by prolonged pipetting or vortexing.
	No or insufficient beads in the reaction.	Use correct stirring settings. Do not pipet more than 96 reactions. Do not use reagent below the minimal level indicated
	Suboptimal reaction conditions.	Ensure proper disruption and heating conditions. Verify correct temperature of the heating block with a thermometer.
Lid of the reaction tube opens during or after heating	Reaction tube not firmly closed.	Ensure that all reaction tubes are firmly closed before heating. Use lid clips for closing the tubes properly. Use a heating unit that enables removal of the tubes without directly touching the tube lids.

2.5 Support

If you have questions or experience any problems with our products, please contact us:

www.hygiena.com/support

Our aim is to provide you with a solution as quickly and effectively as possible. We would also like you to contact us if you have any suggestions for improving the product or in case you would like to use our product for a different application. We highly value your feedback.

3. ADDITIONAL INFORMATION

3.1 General Information

Quality Control

All products are regularly monitored by our quality control. You can find the certificate of analysis (COA) on our website. If you would like to carry out your own quality control, you will find the analysis method described in the certificate.

Waste Disposal

All contaminated and potentially infectious material, like enrichment cultures or food samples, should be autoclaved before disposal and eliminated according to local rules and regulations. For proper disposal of unused chemicals, please refer to the SDS.

Warranty and Disclaimer of Liability

“Limited Warranty” and “Disclaimer of Liability”: Hygiena Diagnostics GmbH warrants that this product is free from defects in materials and workmanship through the expiration date printed on the label and only if the following are complied with:

(1) The product is used according to the guidelines and instructions set forth in the product literature;

(2) Hygiena Diagnostics GmbH does not warrant its product against any and all defects when: the defect is as a result of material or workmanship not provided by Hygiena Diagnostics GmbH; defects caused by misuse or use contrary to the instructions supplied, or improper storage or handling of the product;

(3) All warranties of merchantability and fitness for a particular purpose, written, oral, expressed or implied, shall extend only for a period of one year from the date of manufacture. There are no other warranties that extend beyond those described on the face of this warranty;

(4) Hygiena Diagnostics GmbH does not undertake responsibility to any purchaser of its product for any undertaking, representation or warranty made by any dealers or distributors selling its products beyond those herein expressly expressed unless expressed in writing by an officer of Hygiena Diagnostics GmbH;

(5) Hygiena Diagnostics GmbH does not assume responsibility for incidental or consequential damages, including, but not limited to responsibility for loss of use of this product, removal or replacement labor, loss of time, inconvenience, expense for telephone calls, shipping expenses, loss or damage to property or loss of revenue, personal injuries or wrongful death;

(6) Hygiena Diagnostics GmbH reserves the right to replace or allow credit for any modules returned under this warranty.

Trademarks

3.2 Reference Number

The reference number and original Hygiena Diagnostics GmbH article number: S 400 08.1

3.2 Change Index

Version 1, February 2020:
First version of the manual

Version 2, July 2021:
Steps for optional pre-concentration added for procedures B and D.

Revision A, December 2023:
Rebranding and layout
S 400 08.1 20-5 -> INS-KIT230177-5-REVA

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