foodproof®

Animal Detection 1 LyoKit
Ready Reference Guide

Revision A, November 2023

Product No. KIT230127 (LP), KIT230128 (RP)

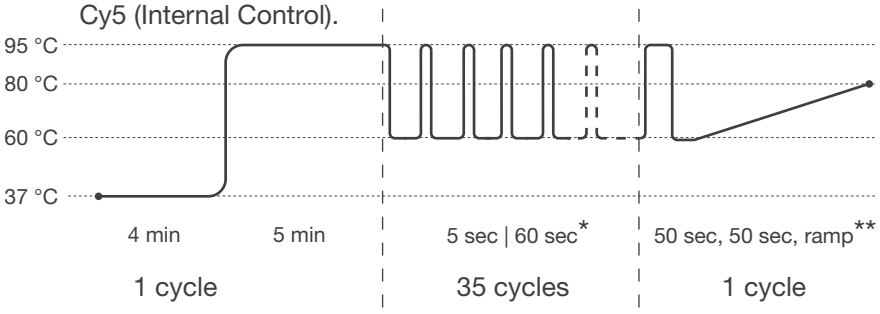
PCR kit for the qualitative detection of porcine, bovine and equine with differentiation of horse, donkey and zebra.

Before starting, it is strongly recommended to read the entire product manual available on our website.

PROGRAM SETUP

Program your real-time PCR instrument before setting up the PCR reactions. Select the following channels:

- FAM (porcine), HEX (bovine), ROX (equine: horse, donkey, zebra) and Cy5 (Internal Control).



Pre-incubation: 1 cycle
Step 1: 37 °C for 4 min
Step 2: 95 °C for 5 min

Amplification: 35 cycles
Step 1 : 95 °C for 5 sec
Step 2*: 60 °C for 60 sec

Melting Curve: 1 cycle
Step 1 : 95 °C for 50 sec
Step 2 : 60 °C for 50 sec
Step 3**: ramp up to 80 °C

* Fluorescence detection

** Fluorescence detection during 60 - 80 °C ramp with 1 measurement/°C

For CFX96™ real time PCR cycler step 2 of melting curve is: 50 °C for 50 seconds. For ABI 7500 Fast, step 2 of melting curve is: 50 °C for 60 seconds, step 3: ramp up to 85 °C. For some real-time PCR instruments, the probe quencher as well as the usage of a passive reference dye has to be specified. This kit contains probes with a non-fluorescent "dark" quencher and no passive reference dye. A Color Compensation is necessary for users of the LightCycler® 480 System: Color Compensation Set 3 (Product No. KIT230005).

DATA INTERPRETATION

Verify results of positive (Control Template) and negative controls (H_2O), before interpreting sample results. Always compare samples to positive and negative control. Review data from each channel and interpret results as described in the table.

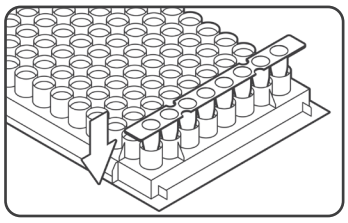
FAM	HEX	ROX	Cy5	Result Interpretation - Amplification
+	+	+	+ or -	Positive for porcine, bovine and equine
-	+	+	+ or -	Positive for bovine and equine
+	-	+	+ or -	Positive for porcine and equine
+	+	-	+ or -	Positive for porcine and bovine
-	+	-	+ or -	Positive for bovine
+	-	-	+ or -	Positive for porcine
-	-	+	+ or -	Positive for equine
-	-	-	+	Negative for porcine, bovine and equine
-	-	-	-	Invalid

Channel	Result Interpretation - Melting Curve		
ROX	Donkey: 64 - 67 °C	Zebra: 68 - 70.5 °C	Horse: 71 - 74 °C

The Control Template contains only donkey and horse. Therefore, two peaks will be displayed. Please use the Control Template as a reference. Melting curve peaks can vary about ± 1 °C depending on PCR instrument used.

PREPARATION OF THE PCR MIX

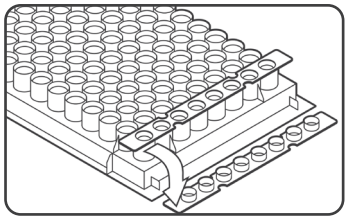
Take appropriate precautions to prevent contamination, e.g., by using filter tips and wearing gloves.



1. PLACE STRIPS IN RACK

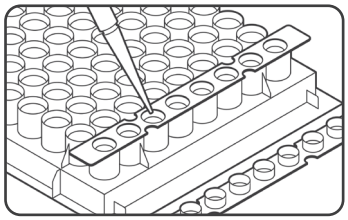
Take needed number of PCR tube strips out of aluminum bag.
Important: close bag tightly afterwards. Place strips in a suitable PCR tube rack.

If needed, gently tap the tubes to move the lyophilized pellets to the bottom of all tubes.



2. DECAP

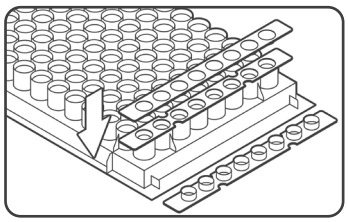
Immediately before filling, carefully open strips and discard caps.
Do not leave open longer than necessary.



3. ADD SAMPLES AND CONTROLS

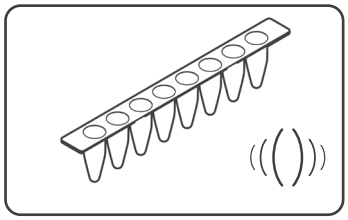
Pipette 25 µL of samples (from Sample Preparation Kit III), Negative Control (colorless cap) or Control Template (purple cap) into respective wells.

For samples prepared with StarPrep Five, use 22 µL PCR-grade water + 3 µL sample for a total volume of 25 µL.



4. SEAL

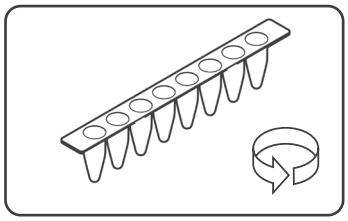
Carefully seal the tubes with the provided 8-cap strips.



5. MIX

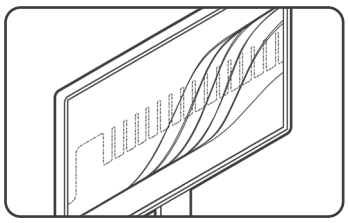
Resuspend pellet after sealing by mixing thoroughly.

Alternatively, resuspend pellet by pipetting up and down multiple times in Step 3.



6. CENTRIFUGE

Briefly spin strips, e.g., 5 seconds at 500 - 1,000 x g, in a suitable centrifuge.



7. START REAL-TIME PCR RUN

Cycle samples as described above.

Place tubes in a vertical, balanced order into the cycler, e.g., two strips can be placed in the first and last column.

foodproof®
Animal Detection 1 LyoKit

KIT230127 / 28
Kit for 96 reactions
Store kit at 2 to 8 °C

For food testing purposes
FOR IN VITRO USE ONLY
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Hygiena® | Camarillo, CA 93012 USA | diagnostics.support@hygiena.com | www.hygiena.com