YOUSEQ

EQUINE ENDOGENOUS CONTROL

qPCR HANDBOOK

For Research Use Only. Not intended for diagnostic use.

CAT NO.: YSL-qP-EndoEquine-FAM/HEX/ROX/CY5

100 reactions

VERSION 1.0

YOUSEQ

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INTENDED USE

This primer/probe mix is designed to be used as an Equine Endogenous Control for a qPCR test. It is designed to be used by trained professionals in a suitable molecular biology laboratory.

SPECIFICITY

The YouSeq Equine Endogenous Control qPCR test is designed to have the broadest detection profile possible. The primers and probes typically have ≥95% homology with all reference data used, from relevant, publicly available databases at the time of design.

The target gene (ACTB) has been demonstrated to have a distinctive sequence making it an ideal target for highly specific detection.

For further information on the detection profile of the product, please do not hesitate to contact our team: support@youseq.com

CONTENTS

Component	Cap Colour	Volume
Equine Endogenous Control primer/probe mix (specified channel)		100 µL

MATERIALS REQUIRED BUT NOT PROVIDED

qPCR MasterMix (Recommended for use in combination with YouSeq Tetra™ MasterMix range: www.youseq.com).

Nucleic acid template.

General laboratory equipment (pipettes, pipette tips, (micro)centrifuge tubes, etc.).

qPCR instrument.

BEST PRACTICE

Decontamination:

Before beginning laboratory work, thoroughly decontaminate any work surfaces and pipettes being used, to eliminate potential contamination.

General use and set-up:

All components should be fully defrosted with contents at the bottom of the tube before opening. To ensure contents are at the bottom, centrifuge or gently tap the tube. After use, reagents should be returned to the freezer.

Once any reagents are resuspended, mark the tick box on the tube for future reference. After this, or after combining reagents, the tube should be pulse vortexed to ensure it is mixed well.

It is advised to set up the plate and reaction mix on ice to minimise artefact formation, which may reduce sensitivity.

When preparing the qPCR reaction mix, it is recommended to incorporate an overage when calculating the total number of reactions to compensate for potential volume losses incurred during pipetting.

Set-up environments:

It is best practice to set up qPCR tubes/plates in two different environments - a clean (no template) lab and PCR (template) lab.

No Template Control(s) (NTC) and Positive Control(s) (PTC) should be included in every run. To reduce contamination, NTCs and

samples can be set up and sealed in a clean lab before moving to the PCR lab.

BENCH SIDE PROTOCOL

qPCR REACTION SET UP

When setting up the qPCR reaction mix, the Equine Endogenous Control primer/probe is designed to be 20X mix.

Example: 1 µL of Equine Endogenous Control primer/probe in a 20 µL total reaction volume.

qPCR AMPLIFICATION PROTOCOL

1. Load the plate/tubes onto the qPCR instrument and set up the required qPCR protocol.
    a. Follow the amplification protocol as per the MasterMix manufacturer's instructions.
    b. The primer/probe mix is optimised to work in combination with YouSeq Tetra™ MasterMix using the protocol in the table below, based on a DNA or RNA sample input.

DNA

Temperature	Time	Number of Cycles
95°C	3 minutes	-
95°C	15 seconds	x 45
60°C 📷	60 seconds	

📷 Collect fluorogenic data through the specified channel during this step.

RNA

Temperature	Time	Number of Cycles
55°C	10 minutes	-
95°C	3 minutes	-
95°C	15 seconds	x 45
60°C 📷	60 seconds	

📷 Collect fluorogenic data through the specified channel during this step.

INTERPRETATION OF RESULTS

The Cq value from the Endogenous Control will vary according to the amount of DNA/RNA in the sample. In conjunction with YouSeq Tetra™ 2X qPCR MasterMix, a Cq value of ≤ 28 indicates a successful extraction has taken place with sufficient equine DNA in the sample. If the signal is later than this, repeating the nucleic acid extraction is advised.

PRODUCT SPECIFICATIONS

Storage

Store at -20C from arrival. The product's shelf life is outlined as an expiry date on the pouch label.

Suitable input material

This product will work well with any source of good quality nucleic acid. Good quality is defined as nucleic acid with high integrity (not degraded). Poor quality input nucleic acid is the biggest cause of test failure.

Regulatory status

This product has been developed for Research Use Only and is not intended for diagnostic use.

Quality Control

In accordance with the YouSeq Ltd ISO EN 13485-certified Quality Management System, each lot of YouSeq Endogenous control primer/probe mix is tested against predetermined specifications to ensure consistent product quality. The primers/probe(s) typically demonstrate ≥95% in silico specificity to their intended target and periodically checked against newly available sequence information to maintain their detection profile.

Technical Assistance

For customer support, please contact:

e-mail: support@youseq.com
phone: +44 (0)333 577 6697

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