Primerdesign

Z-Path-TKP-GPX

Taylorella equigenitalis, Klebsiella pneumoniae and Pseudomonas aeruginosa (TKP)

Kit version: 1

Target regions:

T.equigenitalis (gyrB gene)

K.pneumoniae (phoE)

P.aeruginosa (regA gene)

genesig® PLEX kit

100 tests

GENESIG

Kits by Primerdesign

Product Description

This genesig®PLEX TKP kit detects three pathogenic bacteria (Taylorella equigenitalis, Klebsiella pneumoniae and Pseudomonas aeruginosa) associated with sexually transmitted diseases (STDs) in horses which are important to monitor for breeding.

T. equigenitalis causes contagious equine metritis, an inflammatory disease of the reproductive tract of the mare which usually results in temporary infertility. K. pneumoniae results in endometritis, which requires treatment with antibiotics. P. aeruginosa is an opportunistic, infection of the guttural pouch, reproductive tract, eyes, and lower respiratory tract, especially after antimicrobial therapy.

Specificity

The genesig®PLEX TKP kit is designed for the in vitro detection of T. equigenitalis, K. pneumoniae and P. aeruginosa. The kit is designed to have a broad detection profile. Specifically, the primers will detect over 95% of sequences available on the NCBI database at the time of last review.

The K. pneumoniae assay is predicted to cross-react with other members of the Klebsiella pneumoniae species complex; K. quasipneumoniae, K. variicola, K. quasivariicola and K. africana.

The dynamics of genetic variation mean that new sequence information may become available after the most recent review. If you require further information or have a specific question about the detection profile of this kit, then please send an email to techsupport@primerdesign.co.uk and our team will answer your question.

Kit contents

Quantity	Component	Tube	Cap Colour
1	**T.EQUI/K.PNE/P.AERU primer/probe mix (100 reactions)** FAM labelled, Target: K. pneumoniae VIC labelled, Target: P. aeruginosa ROX labelled, Target: T. equigenitalis Cy5 labelled, Target: Internal extraction control		**BROWN**
1	**T.EQUI/K.PNE/P.AERU positive control template**		**RED**
1	**Internal extraction control DNA**		**BLUE**
2	**oasig® 2X qPCR Master Mix Lyophilised**		**SILVER**
2	**oasig® resuspension buffer** For resuspension of the lyophilised master mix		**BLUE**
1	**Template preparation buffer** For resuspension of the positive control template and Internal extraction control DNA		**YELLOW**
1	**RNase/DNase free water** For resuspension of the primer/probe mix		**WHITE**

Reagents and equipment to be supplied by the user

Real-time PCR Instrument

Extraction kit

This kit is recommended for use with genesig® Easy DNA/RNA Extraction kit or exsig®Mag. However, it is designed to work well with all processes that yield high-quality nucleic acid with minimal PCR inhibitors.

Pipettors and filter tips

Vortex and centrifuge

1.5 ml tubes

qPCR plates or reaction tubes

Kit storage and stability

This kit is stable for shipping at ambient temperature but should be stored at -20°C upon arrival. Once the lyophilised components have been resuspended, they should not be exposed to temperatures above -20°C for longer than 30 minutes at a time and unnecessary repeated freeze/thawing should be avoided. The kit is stable for six months from the date of resuspension under these circumstances.

Primer Design Ltd does not recommend using the kit after the expiry date stated on the pack.

Suitable sample material

This kit can be used with all types of samples from various origins. Please ensure that the extracted nucleic acid samples are suitable in terms of purity, concentration, and DNA integrity. An internal extraction control is provided to test for non-specific PCR inhibitors.

Dynamic range of test

Under optimal PCR conditions the kit can achieve priming efficiencies between 90-110% and detect less than 100 copies of target template.

Principles of the test

Real-time PCR

Individual primer and probes designed for each target have been combined into one reaction and these can be detected through the different fluorescent channels as described in the kit contents.

The assay consists of primers and labelled probes in a single test to produce signal for K. pneumoniae in the FAM channel, for P. aeruginosa in the VIC channel and for T. equigenitalis in the ROX channel. The assay includes an internal extraction control DNA, which may be added to the nucleic acid extraction system (not provided) to prove efficient DNA extraction, detect PCR inhibition and confirm the integrity of the PCR run. The internal extraction control assay (which is from a non-biologically relevant, exogenous source) is present in the kit primer/probe mix and the probe is labelled with the Cy5 fluorophore.

The primer and probe mix provided exploits the so-called TaqMan® principle. During PCR amplification, forward and reverse primers hybridize to the target DNA. A fluorogenic probe is included in the same reaction mixture which consists of a DNA probe labelled with a 5′-dye and a 3′-quencher. During PCR amplification, the probe is cleaved, and the reporter dye and quencher are separated. The resulting increase in fluorescence can be detected on a range of qPCR platforms.

Positive control

For a positive control, the kit contains a single positive control that contains templates for K. pneumoniae, P. aeruginosa and T. equigenitalis. The kit positive control will give a K. pneumoniae signal through the FAM channel, a P. aeruginosa signal through the VIC channel and a T. equigenitalis signal through the ROX channel when used with the T.EQUI/K.PNE/P.AERU primer/probe mix. Each time the kit is used, at least one positive control reaction must be included in the run. A positive result indicates that the primers and probes for detecting the target genes worked properly in that particular experimental scenario. If a negative result is obtained the test results are invalid and must be repeated. Care should be taken to ensure that the positive control does not contaminate any other kit component which would lead to false-positive results. This can be achieved by handling this component in a post-PCR environment. Care should also be taken to avoid cross-contamination of other samples when adding the positive control to the run. This can be avoided by sealing all other samples and negative controls before pipetting the positive control into the positive control well.

Negative control

To validate any positive findings a negative control reaction should be included every time the kit is used. For this reaction, the RNase/DNase free water should be used instead of template. A negative result indicates that the reagents have not become contaminated while setting up the run. It is also known as a No Template Control or NTC.

Internal DNA extraction control

When performing DNA extraction, it is often advantageous to have an exogenous source of DNA template that is spiked into the lysis buffer. This control DNA is then co-purified with the sample DNA and can be detected as a positive control for the extraction process. Successful co-purification and

qPCR for the control DNA also indicates that PCR inhibitors are not present at a high concentration.

Within the Multiplex primer/probe mix are primers and probes to detect the exogenous DNA using qPCR. The primers are present at PCR limiting concentrations which allows multiplexing with the target sequence primers. Amplification of the control DNA does not interfere with detection of the target DNA even when present at low copy number. The Internal control is detected through the Cy5 channel and gives a Cq value of 28+/-3.

Resuspension protocol

To minimise the risk of contamination with foreign DNA, we recommend that all pipetting is performed in a PCR clean environment. Ideally this would be a designated PCR lab or PCR cabinet. Filter tips are recommended for all pipetting steps.

1. **Pulse-spin each tube in a centrifuge before opening.**
   This will ensure lyophilised primer/probe mix is in the base of the tube and is not lost upon opening the tube.

2. **Resuspend the primer/probe mix in the RNase/DNase free water supplied, according to the table below:**
   To ensure complete resuspension, allow primer/probe mix to rehydrate for 10 minutes at room temperature. Vortex the tube thoroughly, followed by pipetting up and down 10 times. Failure to mix well can produce poor kit performance.

Component – resuspend in water	Volume
Pre-PCR pack	
T.EQUI/K.PNE/P.AERU primer/probe mix (**BROWN**)	110µl

3. **Resuspend the positive control template in the template preparation buffer supplied, according to the table below:**
   To ensure complete resuspension, vortex the tube thoroughly.

Component – resuspend in template preparation buffer	Volume
Pre-PCR heat-sealed foil	
Internal extraction control DNA (**BLUE**)	600µl
Post-PCR heat-sealed foil	
T.EQUI/K.PNE/P.AERU positive control template (**RED**)*	500µl

*** This component contains high copy number template and is a VERY significant contamination risk. It must be opened and handled in a separate laboratory environment, away from the other components.**

4. **Resuspend the lyophilised Master Mix in oasig® resuspension buffer, according to the table below:**

Component – resuspend in oasig resuspension buffer	Volume
oasig® 2X qPCR Master Mix Lyophilised (SILVER)	525µl

DNA extraction

The internal extraction control DNA can be added to either lysis/extraction buffer or to the sample once it has been resuspended in lysis buffer.

DO NOT add the internal extraction control DNA directly to the unprocessed biological sample as this will lead to degradation and a loss in signal.

1. **Add 4µl of the Internal extraction control DNA (BLUE) to each sample in DNA lysis/extraction buffer per sample.**

2. **Complete DNA extraction according to the manufacturer's recommended protocols.**

qPCR detection protocol

For optimum performance and sensitivity
All pipetting steps and experimental plate set up should be performed on ice. After the plate is prepared, proceed immediately to the amplification protocol. Prolonged incubation of reaction mixes at room temperature can lead to PCR artifacts that reduce the sensitivity of detection.

1. **For each DNA sample prepare a reaction mix according to the table below:**
   Include sufficient reactions for positive and negative controls.

Component	Volume
oasig® 2X qPCR Master Mix Lyophilised (**SILVER**)	10µl
T.EQUI/K.PNE/P.AERU primer/probe mix (**BROWN**)	1µl
RNase/DNase free water (WHITE)	4µl
Final volume	**15µl**

2. **Pipette 15µl of this mix into each well according to your qPCR experimental plate set up.**

3. **Pipette 5µl of DNA sample into each well according to your experimental plate set up.**
   For negative control wells use 5µl of RNase/DNase free water (WHITE). The final volume in each well is 20µl.

4. **Pipette 5µl of positive control template into each well according to your plate set up.**
   The positive control contains templates for K. pneuomiae, P. aeruginosa and T. equigenitalis (**RED**). The final volume in each well is 20µl.

qPCR amplification protocol

Amplification conditions using oasig® 2X qPCR Master Mix.

	Step	Time	Temp
	Enzyme activation	2 min	95 ºC
Cycling x50	Denaturation	10 s	95 ºC
	DATA COLLECTION *	60 s	60 ºC

* Fluorogenic data should be collected during this step through the FAM, VIC, ROX and Cy5 channels.

Interpretation of results

Positive control

The positive control well should give an amplification plot through the FAM channel (K. pneumoniae), the VIC channel (P. aeruginosa) and the ROX channel (T. equigenitalis). There is no internal control template within the positive control so the Cy5 channel should give no signal (flat amplification plot). The positive control signals indicate that the kit is working correctly to detect each target.

No template control (NTC)

The NTC should give a flat line (flat amplification plots) through all channels. Signals in the NTC indicate cross contamination during plate set up.

Internal DNA extraction control

The Cq value obtained with the internal control will vary significantly depending on the extraction efficiency, the quality and quantity of DNA added to the PCR reaction and the individual machine settings. Cq values of 28±3 are within the normal range. When amplifying a target sample with a high genome copy number, the internal extraction control may not produce an amplification plot. This does not invalidate the test and should be interpreted as a positive experimental result.

Sample data

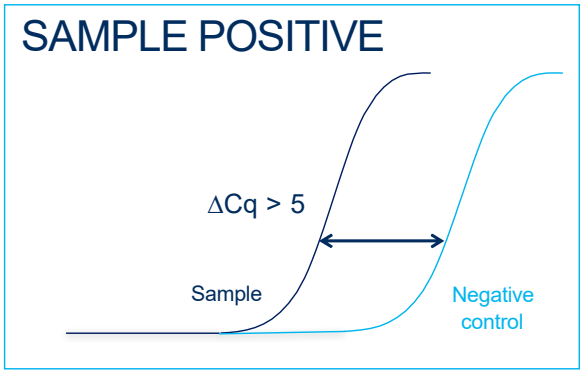
Presence of the targets are detected in the channels indicated in the kit contents section. Positive signals indicate positive tests for those targets. It may be possible for samples to contain multiple targets, therefore positive results in the FAM, VIC and ROX channels may be present.

Summary of data interpretation

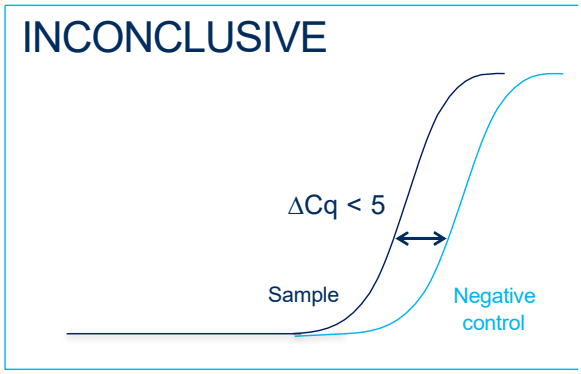
Target (FAM/VIC/ROX)	Internal extraction control (Cy5)	Positive Control	Negative Control	Interpretation
FAM +	**+ / -**	**+**	**-**	**K.pneumoniae POSITIVE RESULT**
VIC +	**+ / -**	**+**	**-**	**P.aeruginosa POSITIVE RESULT**
ROX +	**+ / -**	**+**	**-**	**T.equigenitalis POSITIVE RESULT**
-	**+**	**+**	**-**	**NEGATIVE RESULT**
+ / -	**+ / -**	**+**	**≤35**	**EXPERIMENT FAILED Due to test contamination**
+ / -	**+ / -**	**+**	**>35**	*
-	**-**	**+**	**-**	**SAMPLE PREPARATION FAILED**
+ / -	**+ / -**	**-**	**+ / -**	**EXPERIMENT FAILED**

Positive control template (**RED**) is expected to amplify between Cq 16 and 23. Failure to satisfy this quality control criterion is a strong indication that the experiment has been compromised.

* Where the test sample is positive, and the negative control is positive with a Cq >35, the sample must be reinterpreted based on the relative signal strength of the two results:



If the sample amplifies > 5 Cq earlier than the negative control then the sample should be reinterpreted (via the table above) with the negative control verified as negative.



If the sample amplifies < 5 Cq earlier than the negative control then the positive sample result is invalidated and the result should be determined inconclusive due to test contamination. The test for this sample should be repeated.

Notices and disclaimers

This product is developed, designed and sold for research purposes only. It is not intended for human diagnostic or drug purposes or to be administered to humans unless clearly expressed for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. During the warranty period, Primer Design Ltd genesig® detection kits allow precise and reproducible data recovery combined with excellent sensitivity. For data obtained by violation of the general GLP guidelines and the manufacturer's recommendations the right to claim under guarantee is expired. PCR is a proprietary technology covered by the several US and foreign patents. These patents are owned by Roche Molecular Systems Inc. and have been sub-licensed by PE Corporation in certain fields. Depending on your specific application you may need a license from Roche or PE to practice PCR. Additional information on purchasing licenses to practise the PCR process may be obtained by contacting the Director of Licensing at Roche Molecular Systems, 1145 Atlantic Avenue, Alameda, CA 94501 or Applied Biosystems business group of the Applera Corporation, 850 Lincoln Centre Drive, Foster City, CA 94404. In addition, the 5' nuclease assay and other homogeneous amplification methods used in connection with the PCR process may be covered by US Patents 5,210,015 and 5,487,972, owned by Roche Molecular Systems, Inc, and by U.S. Patent 5,538,848, owned by The Perkin-Elmer Corporation.

Trademarks

genesig® is a registered trademark of Primer Design Ltd.
oasig® is a trademark of Primer Design Ltd.
exsig®Mag is a trademark of Primer Design Ltd.

The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG. TaqMan® is a registered trademark of Roche Molecular Systems, Inc., The purchase of the Primer Design Ltd reagents cannot be construed as an authorization or implicit license to practice PCR under any patents held by Hoffmann-LaRoche Inc.