



Ready To Use PCR Reagents

FELINE POLYCYSTIC KIDNEY DISEASE

Cat. No. 60FPK100
INSTRUCTION MANUAL

I. Intended Use

FPKD PCR Ready to Use PCR Reagents are intended for a successful amplification of the PKD1 gene from gDNA, where the known mutation "C > A" at position 3284 leads to the Feline Autosomal Dominant Polycystic Kidney Disease. All reagents are ready to use for a successful PCR amplification and a complete protocol of the digestion and mutation identification steps is included in the package.

II. General Information

Each package contains **Reaction Negative Control** (Tube A). Another 4 tubes are components needed for PCR amplification. Tube **B** contains **FPKD-PCR mix**, Tube **C** contains **FPKD Activation Buffer**, Tube **D** contains the **Positive Control**, Tube **G** contains a **FPKD Wild Type Control**. Tube **H** contains **FPKD 6X Gel Loading Buffer**. Each PCR set up should include 4 reaction vials; each vial should be added with: **5µl FPKD-PCR mix**, **10µl FPKD Activation Buffer** and **5µl gDNA product / Positive Control/ Reaction Negative Control/ FPKD Wild type Control**. Following the addition and mixing of all the above ingredients, the reaction vials are placed in thermal cycler for amplification according to the program detailed in the Step by Step chapter (see section VIII). At the end of the program an Enzymatic Digestion should be performed, differentiating the products visualized on 1.5% Agarose gel.

III. Description Of The Disease

Feline Autosomal Dominant Polycystic Kidney Disease (FPKD) is an inherited disease that has been recognized mostly in Persian and Persian related cats. The disease is characterized by cysts of various sizes that occur in the renal cortex and medulla and occasionally in the liver, pancreas and uterus. Affected cats remain clinically normal for most of their lives, and renal failure occurs only later in life (usually after 7 years of age). Although the dysfunction of the kidney does not occur until mid to older age, the cysts are present from birth. The size of cysts can vary from less than 1 mm in young animals to greater than 1 cm in size in older animals. The number of cysts also varies from one to more than 200 per kidney.

IV. Diagnosis Of The Disease

FPKD can be diagnosed by ultrasound with high level of confidence. In 2004 Lyons et al. published identification of a mutation "C > A" at position 3284 resulting in FPKD. The "C > A" transversion was found in heterozygous state in Persians and other breeds out-crossed with Persians. A presently available DNA test can identify cats that will develop PKD in future using polymerase chain reaction (PCR) amplification of product size 559 on exon 29. The mutation causes a restriction enzyme site alteration for MLY1, producing two fragments of 316 and 243 bp. Cats with wild type gene PKD1 do not have any stop mutation, resulting in one non-digested amplification product in size 559bp after restriction fragment length polymorphism (RFLP).

Note: FPKD is an autosomal dominant disease, and no homozygote mutants have been reported. FPKD positive phenotype represent a combined genotype of one normal allele (with result at **559bp**) and the mutated allele (with result at **316bp** and **243bp**).

V. Kit contents - Sufficient for 48 tests

Tube A	Reaction Negative Control
Tube B	FPKD-PCR mix (Green cap)
Tube C	Specific FPKD Activation Buffer (Blue cap)
Tube D	Specific FPKD Positive Control (Red cap)
Tube G	FPKD Wild Type Control (Red cap)
Tube H	FPKD 6X Gel Loading Buffer (Green cap)
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VI. Essentials Not Included

High purity gDNA isolation Kit (with Proteinase K)
MLY1 Restriction Enzyme (New England Biolabs cat# R0610).
Buffer 4 (B7004S NEB), BSA (B9001S NEB).
RNAase free PCR reaction vials.
PCR Thermo-Cycler.
5-10µl, 100µl Pipettes and filter tips.
Micro-centrifuge.
Heating bath or heating block.
Agarose; DNA size marker.
Microwave for Agarose casting.
Horizontal Mini-Electrophoresis chamber, Comb and power pack.
TBE /TAE Buffer and Ethidium Bromide (EB).
UV Transilluminator (254nm for EB).

VII. Storage And Handling

- Store at 4°C for 6 months or at -20°C for two years.
- Use gloves and maintain clean working conditions.
- Avoid spillage and cross contamination of solutions.
- Change tips between reagents and between reaction vials.
- Do not mix reagents from different batches.
- Always treat samples with precaution, and dispose as biological material.
- Remember that Ethidium Bromide is hazardous, and use the UV transilluminator carefully.
- It is recommended to incinerate the contents after use.

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VIII. Step By Step Protocol

Notes:

- The Genomic DNA should be cleaned using an Extraction/Purifying kit, which includes **Proteinase K**, before PCR amplification. (A DNA product extracted with any Ready To Use PCR Reagents will not work)!
- This test needs an addition of MLY1 restriction enzyme of New England BioLabs (Cat:R0610) in step 5.

(1) Into a clean reaction vial add: **5µl FPKD-PCR mix** (Tube B), **5µl of the gDNA extraction product** and **10µl** of the specific **FPKD-Activation Buffer** (Tube C). Mark each reaction vial properly to avoid mistakes.

(2) Into another clean reaction vial add **5µl FPKD-PCR mix** (Tube B), **5µl of the Positive Control** (Tube D) and **10µl** of the specific **FPKD Activation Buffer** (Tube C). Mark this vial as Positive Control reaction.

(3) Into another clean reaction vial add **5µl FPKD-PCR mix** (Tube B), **5µl of the Wild Type Control** (Tube G) and **10µl** of the specific **FPKD Activation Buffer** (Tube C). Mark this vial as Wild Type Control reaction.

(4) Into another clean reaction vial add **5µl FPKD-PCR mix** (Tube B), **5µl of the Reaction Negative Control** (Tube A) and **10µl** of the specific **FPKD Activation Buffer** (Tube C). Mark this vial as Negative Control reaction vial.

(5) Mix well each reaction vial and place in the thermal cycler for amplification.

ADS Specific PCR Program:

- 95°C for 3 minutes**
- 40 cycles of**
- 95°C for 45 seconds**
 - 58°C for 45 seconds**
 - 72°C for 45 seconds**
- End cycles**
- 72°C for 3 minutes**
 - Stop at 8°C**

(6) If not used immediately, store PCR products in 4°C until enzymatic digestion and application on Agarose.

IX. Reading And Interpreting The Results

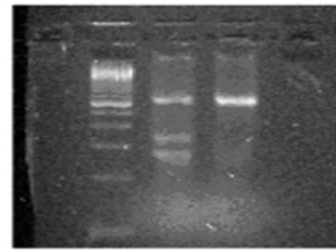
Perform the enzymatic digestion reaction with each PCR reaction product.

Enzymatic Digestion:

18µl of the PCR product.
3µl of buffer 4 (B7004S NEB).
3µl BSA (B9001S NEB).
1.5µl MLY1 (R0610L NEB).
Incubate for 1.5 hours at 37°C.

- Visualize PCR products on 1.5% Agarose gel, along with a size marker (see Fig. 1).
- Mark each well and load the whole content of each reaction vial into the relevant wells.
- Into each reaction vial, add **6µl 6X Gel Loading Buffer** (Tube H), before loading onto Agarose gel.
- No band should be detected at the Negative Control lane.
- The Positive Control should yield three bands of **559bp**, **316bp** and **243bp**.
- A single band of **559bp** should be detected in healthy – wild type patient,
- A patient that has the mutation should yield three bands of **559bp**, **316bp** and **243bp**.

Fig. 1 - Visualization of the PCR product.



Lane 1: Size Marker; Lane 2: FPKD Positive (heterozygote)
Lane 3: Wild Type (homozygote); Lane 4: Negative control

X. Limitations And Troubleshooting

- For *in vitro* use only. Do not use internally or externally in humans or animals.
- A false positive result may occur, even if precaution has been taken. To eliminate inconclusive results, always use the Negative Control in each PCR set.
- This Kit provides detection of the known mutation leading to PKD but there is always an option that a different mutation exists and in such cases, the kit would yield a false negative result.

XI. References

- Helps C et al. (2007) Correlation of the feline PKD1 genetic mutation with cases of PKD diagnosed by pathological examination. *Exp Mol Pathol.* 83(2):264-8. Epub 2007 May 4.
- Lyons LA et al. (2004) Feline polycystic kidney disease mutation identified in PKD1. *J. Am. Soc. Nephrol.* 15: 2548–2555.
- Young AE (2005) Feline polycystic kidney disease is linked to the PKD1 region. *Mamm Genome.* 16(1):59-65.

For further information and assistance please contact your local distributor or Biogal Galed Labs. Directly by e-mail: info@biogal.co.il or by tel: 972-4-9898605 / fax: 972-4-9898690.