AmpliSNiP *PKD1 – C3284A* (qPCR)

Diagnostic kit for analysis of the **C3284A** polymorphism located in the ***PKD1*** gene by the **genotyping** method

Cat. No.	*Quantity*
SNP024-50	**50 assays**

The diagnostic kit is intended for professional use in specialised clinical, diagnostic and research laboratories. We strongly recommend that you read the user manual attached to the diagnostic kit before first use.

Amplicon Sp. z o.o.. • ul. Klecińska 125, 54-413 Wrocław, Poland
VAT No. PL8943052339 • +48 739 223 268 • contact@amplicon.pl
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TRANSPORT

The **AmpliSNiP *PKD1* – C3284A (qPCR)** kit is transported in dry ice. Immediately unpack the kit after delivery. Immediately inform the manufacturer if the tape securing the transport box or the seal of the commercial packaging are damaged or there is no dry ice left in the styrofoam transport box.

KIT CONTENTS

Component	*Description*	**SNP024-50** *50 assays*	*Tube lid colour*
RM	Reaction Mixture	1 × 550 µL	Green
PC-G	Positive Control	1 × 300 µL	Red
PC-T	Positive Control	1 × 300 µL	Red
NC	Negative Control	1 × 300 µL	Blue
WATER	Water	1 × 1500 µL	White

STORAGE AND TRANSPORTATION CONDITIONS

- Store the kit components at -20°C.
- **AVOID THE EXPOSURE OF THE RM COMPONENT TO THE LIGHT**.
- Avoid repeated freezing/thawing cycles of kit components (especially the RM component). The kit is stable after 10 repeated freezing/thawing cycles.
- The kit is stable under recommended storage conditions until the expiry date stated on the label.
- Do not use kit components after the expiry date.

PRODUCT DESCRIPTION

Application

The **AmpliSNiP *PKD1* – C3284A (qPCR)** kit is designed to allow the analysis of the C3284A polymorphism located in the *PKD1* gene, which is associated with development of the polycystic kidney disease in cats. The allelic variant, in which the G nucleotide is replaced by the T nucleotide, encodes a dysfunctional version of the protein, which is responsible for the development of the disease in cats. The diagnostic kit is intended for *in vitro* diagnostics. The kit is a qualitative type: it is used to determine the allelic variants of the feline gene tested.

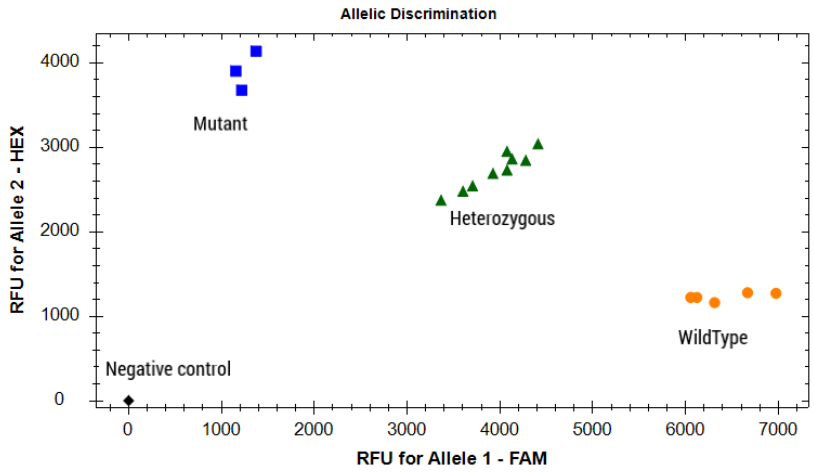
Method principle

The **AmpliSNiP *PKD1* – C3284A (qPCR)** kit relies on the genotyping method. The kit contains two probes designed to bind specifically to a sequence containing a different nucleotide at the polymorphic site. The probe labelled with FAM day is complementary to the sequence containing the G nucleotide, while the probe labelled with the HEX dye to the sequence containing the T nucleotide at the polymorphic site. During reaction, these probes compete with each other for binding to the DNA sequence. The probe with 100% homology to the DNA strand binds to it preferentially. As the reaction progresses, a strong signal appears due to dye release from the fully homologous probe and a weak signal is observed for the alternative one. The genotype of the sample is determined by comparing the signal intensity ratio between the two channels (FAM and HEX)*. For homozygous samples, a strong signal is obtained in one channel and weak in the other one. For heterozygous samples which contain binding sites for each probe, an intermediate signal is obtained in both channels. (Fig. 1).

* The determination of individual variants (alleles) is based on the final measurement of dye fluorescence intensity. Strong fluorescent signals for each allele provide an easy distinction between two homozygous genotypes and one heterozygous

genotype, due to the formation of three clear clusters in the allelic discrimination graph (Fig. 1). The software in most Real Time PCR devices performs such analyses automatically.

Fig. 1: Allelic discrimination graph.



The **AmpliSNiP *PKD1* – C3284A (qPCR)** kit contains one **RM** component which allows determination of the feline genotype (GG/TT/TG). In addition, the kit contains two different **PC** components (positive controls) which contain DNA molecules encoding the genotypes. To ensure clear cluster separation, the number of positive control molecules added to the reaction mixture should be similar to the number of the molecules of genomic DNA.

ADDITIONAL INSTRUMENTS AND MATERIALS

The **AmpliSNiP *PKD1* – C3284A (qPCR)** kit allows a complete diagnostic procedure (analysis of the C3284A polymorphism within the *PKD1* gene in the samples of feline origin) **only** in conjunction with the kit for DNA extraction from the sample and the Real Time PCR device.

NOTE 1: The **AmpliSNiP *PKD1* – C3284A (Real Time PCR)** kit is compatible with Real Time PCR devices having the following parameters:
• detection of HEX and FAM fluorescent dyes;
• possibility to adjust the heating and cooling rate of the heating block (known as the RAMP RATE parameter) to 2,0°C;
A full list of compatible Real Time PCR devices can be found on the manufacturer's website.

NOTE 2: The **AmpliSNiP *PKD1* – C3284A (Real Time PCR)** kit does not contain the ROX reference dye.
For devices requiring normalisation, the ROX dye should be added to the RM component at the appropriate concentration. The ROX dye is not included in the kit.

TECHNICAL SPECIFICATION

Quality control	Complies with ISO 13485: Medical devices - Quality management systems
Optimal amount of DNA added to the reaction	to 100 ng

DNA EXTRACTION

For DNA extraction and purification the silica-based DNA extraction kits are recommended. These kits ensure good-quality of DNA samples. DNA samples should be stored at 2–8°C (short storage period), at -20°C or at a lower temperature (long storage period). The amount of the material required depends on DNA extraction method.

NOTE 3: Samples should be collected in sterile tubes. Prior to DNA extraction, samples must be stored for a period and under conditions that guarantee the stability of the feline genetic material.

NOTE 4: Other extraction methods can cause contamination of the DNA samples with compounds that significantly reduce the efficiency of the PCR reaction. This leads to lower sensitivity of the assay and, in extreme situations, complete inhibition of DNA amplification.

REAL TIME PCR REACTION

1. Determine the number of DNA samples to be analysed (**n**).
2. Thaw the kit components. After thawing, mix the tubes thoroughly and briefly spin them.
   Store thawed components at 2–8°C or in ice.
   AVOID THE EXPOSURE OF THE RM COMPONENT TO THE LIGHT.
3. Determine the amount of the DNA sample added to the reaction (**x** µL).

   NOTE 5: Optimal amount of DNA added to the reaction should not exceed 100 ng. A large amount of genomic DNA added to the PCR reaction mixture can lead to non-specific reactions.

4. Mix carefully (**n** + 4) × 11 µL of the **RM** component and (**n** + 4) × (9 - **x**) µL of water.
5. Add (20 - **x**) µL of the mixture to **n** + 3 reaction tubes.
6. Add **x** µL of the DNA samples to **n** reaction tubes. Add **x** µL of the **NC** component (negative control) to one of the three remaining reaction tubes and **x** µL of the **PC** components (**PC-G** and **PC-T**) to the other two ones.
7. Close the reaction tubes and centrifuge them briefly.
8. Place the reaction tubes into the reaction device and run the amplification program according to the following amplification profile. In addition, in the device software, set the heating and cooling rate of the heating block (the RAMP RATE parameter) to 2.0°C.

Step	Temperature	Time	Data Collection	Cycles
Initial denaturation	95°C	5 min		1
Denaturation	95°C	10 s	FAM and HEX	45
Amplification	58°C	25 s		

NOTE 6: It is recommended that you prepare the Real Time PCR reaction in the laminar chamber to reduce the risk of reaction mixture contamination.

RESULT INTERPRETATIONS

The data can be visualised on an allelic discrimination scatter plot using the software provided with the Real Time PCR device. The software, based on RFU values, automatically assigns the genotype to an unknown sample. One axis will show the data from the final fluorescence intensity measurement for the FAM channel, whereas fluorescence intensity for the HEX channel will be shown on the other axis. Each point on the graph represents data for an individual reaction tube (Fig. 1).

The analysis can also be performed by comparing fluorescence intensity on amplification plot. For this, the amplification curves for both channels should be visualised. In the case of homozygotes, there is a strong signal increase observed for one fluorophore in relation to the signal increase for the other one (Fig. 2). In the case of heterozygote, an intermediate signal for both fluorophores is obtained (Fig. 3).

Fig. 2: Amplification curves for homozygous versions of the gene assayed

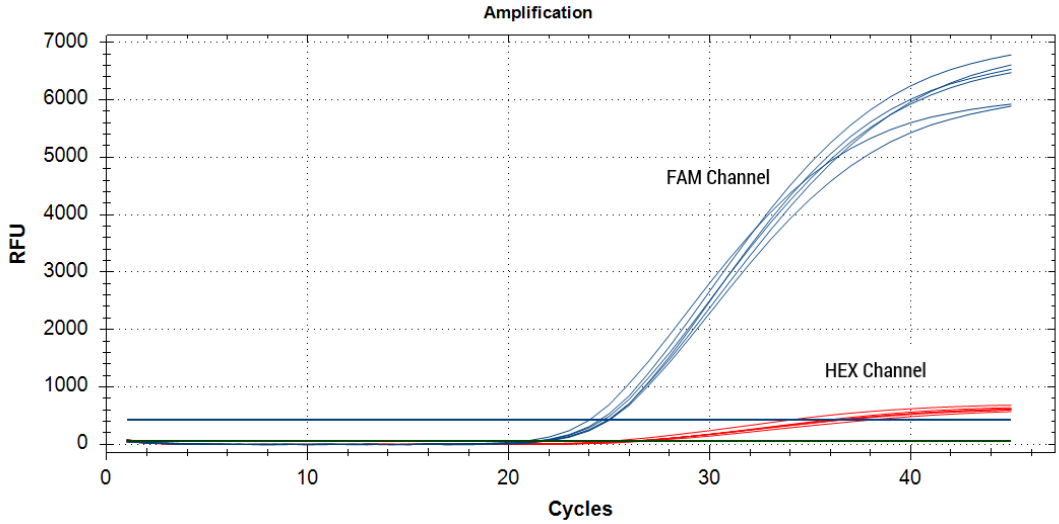
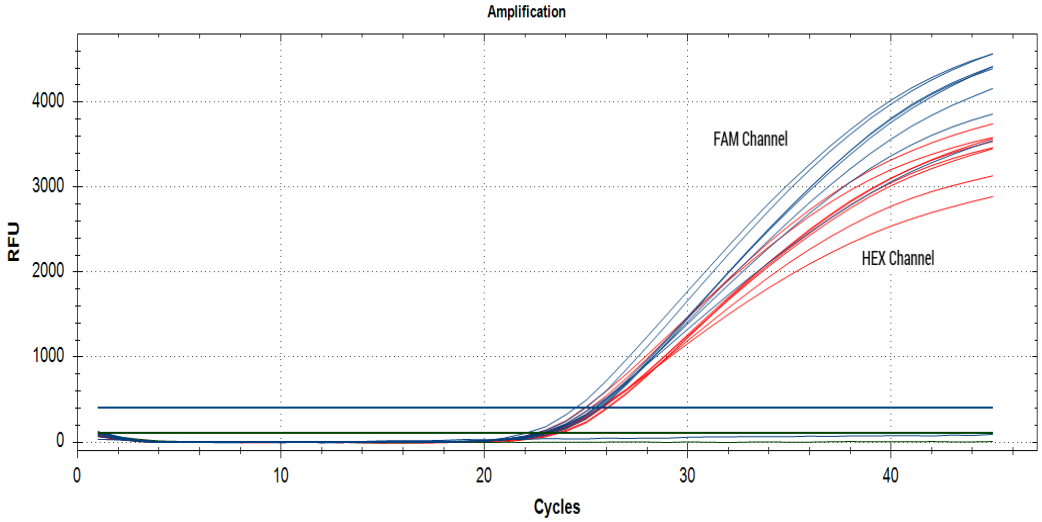


Fig. 3: Amplification curve for heterozygous versions of the gene assayed



ANALYSIS EVALUATION

Step I: Analysis of reactions with control components

Analyse reactions with the **PC** components and **NC** component to check the proper course of the Real Time PCR reaction.

Control	FAM (allele 1)	HEX (allele 2)	Result
PC-G	+	-	Valid
PC-T	-	+	Valid

- means no visible increase in fluorescence or a slight increase in comparison with the alternative positive control
+ means a significant increase in fluorescence compared to an alternative positive control

Control	FAM (allele 1)	HEX (allele 2)	Result
NC	-	-	Valid: no contamination of genomic DNA

- means no PCR product amplification and no fluorescence increase

If you obtain correct results for all control reactions, go to Step II of the analysis.

Step II: Determination of the *PKD1* gene version in the samples

For each sample compare the ratio between the signal recorded in the FAM channel and signal recorded in the HEX channel manually or with the software. Then determine the genotype of the DNA samples.

	FAM (allele 1)	HEX (allele 2)	Result
1	+	-	Valid: homozygote for allele GG
2	-	+	Valid: homozygote for allele TT
3	+	+	Valid: heterozygote GT

- means no visible increase or a slight increase in fluorescence
+ means a marked increase in fluorescence

NOTE 6: No signal on each of the channels means an invalid reaction (no added DNA, too low concentration of DNA or the presence of inhibitors in the sample)

ADDITIONAL REMARKS

- The TaqMan® probes have dark quenchers that do not generate additional fluorescent signals. For devices which require it (e.g. RotorGene 6000), select the **None** option as the quencher type.
- The Real Time PCR device should be calibrated (color compensation should be carried out) for fluorescent dyes.
- The total volume of the reaction mixture is 20 µL. Change of this volume may have a significant impact on some test parameters, including the detection limit.

WARNINGS AND PRECAUTIONS

- Take special care during DNA extraction. The biological material is considered as **potentially infectious material.**
- DNA extraction and the analysis of the C3284A polymorphism located in the *PKD1* gene should be done by properly trained personnel in a professional laboratory which is allowed to work with infectious material. Special attention should be paid to prevent cross-contamination of the DNA samples during DNA extraction.
- To minimise false results, adhere to the following rules:
  - If possible, designate the separate stands for DNA extraction as well as preparation and performance of the Real Time PCR reaction;
  - Change protective clothing and gloves between the stages of DNA extraction and preparation of the Real Time PCR reaction;
  - Bench surfaces should be washed with DNA removing/destructive agents;
  - Use only sterile filter tips for automatic pipettes;
  - The **PC** components should be added to the reaction mixture as the **last ones**. Before adding them, if possible, close the tubes with the DNA samples and **NC** component;
- Do not eat, drink or smoke while using the kit;
- Wash your hands immediately after work with the kit;
- After contact of the skin or eyes with the kit components, gently wash them with water;
- It is recommended that you dispose of the kit after its expiry date or of any unused kit components as a laboratory waste.

TROUBLESHOOTING

Real Time PCR reaction

Problem			Possible cause	Suggestions
	FAM	HEX		
Incorrect fluorescence reading for positive control (**PC-G** component)	-	+	The **PC-T** component instead of the **PC-G** component was added to the reaction.	Repeat the Real Time PCR reaction for the positive control.
	-	-	No **PC** component added, the **NC** component instead of the **PC** component was added to the reaction or the **RM** component is of poor quality.	Repeat the Real Time PCR reaction for the positive control. If you receive an incorrect result again, assume the poor quality of the **RM** component (improper storage, improper transport conditions or exceeding the expiry date).
Incorrect fluorescence reading for positive control (**PC-T** component)	+	-	The **PC-G** component instead of the **PC-T** component was added to the reaction.	Repeat the Real Time PCR reaction for the positive control.
	-	-	No **PC** component added, the **NC** component instead of the **PC** component was added to the reaction or the **RM** component is of poor quality.	Repeat the Real Time PCR reaction for the positive control. If you receive an incorrect result again, assume the poor quality of the **RM** component (improper storage, improper transport conditions or exceeding the expiry date).

Incorrect fluorescence reading for negative control	-	+	The **PC** component instead of the **NC** component was added to the reaction. Contamination of the **RM** or **NC** component.	Repeat the Real Time PCR reaction for the negative control with **WATER** instead of the **NC** component. If you receive an incorrect result again, assume the contamination of the **RM** component.
	+	-		
	+	+		
Incorrect fluorescence reading for the tested sample	-	-	No DNA sample added to the reaction. Low DNA extraction yield and/or Real Time PCR inhibitors present. Too high DNA concentration. Poor quality of the **RM** component.	Measure the concentration of the DNA sample and, if necessary, repeat the reaction with less DNA added to the reaction. In the absence of amplification for the positive controls, assume the poor quality of the **RM** component. In case of amplification observed for positive controls and no amplification for DNA samples assayed, assume contamination of the DNA samples with PCR inhibitors or low efficiency of nucleic acid extraction.

CUSTOMER SERVICE

- Any problems or irregularities that arise during the use of the diagnostic kit can be reported by phone or email.
- Orders for the **AmpliSNiP (qPCR)** kits can be sent by email.

CONTACT

Support and customer care

+48 739 223 268
contact@amplicon.pl