

Read the instruction leaflet carefully prior to testing. For professional use only.

Cat No.: GLVC031



## Canine Anaplasma spp. Real Time PCR Kit

**Canine Anaplasma Real Time PCR Kit** for the qualitative/quantitative detection of nucleic acid from Whole blood, serum, Plasma tissue of infected animals.

### INTENDED USE

**Canine Anaplasma spp. Real Time PCR Kit** is designed for the qualitative/quantitative detection of nucleic acid from Whole blood, serum, Plasma tissue of infected animals. The results can be used to assist diagnosis of infected animals with Canine Anaplasma infection, and provide molecular diagnostics. The test results of this product are for clinical reference only and should not be used as the only standard for clinical diagnosis. It is recommended to conduct a comprehensive analysis by combining the test results with clinical symptoms and other laboratory tests.

### INTRODUCTION

The bacteria *Anaplasma platys* and *Anaplasma phagocytophilum* are tick-borne agents that cause canine vector-borne disease. *A. phagocytophilum* causes canine granulocytic anaplasmosis, *A. platys* canine cyclic thrombocytopenia. Non-specific clinical signs such as lethargy, inappetence/anorexia, splenomegaly and fever are the most common clinical signs reported in *A. phagocytophilum* infection. Despite descriptions which include fever, lethargy, anorexia, pale mucous membranes, petechiae and lymph adenomegaly, it is still controversial whether *A. platys* infection is responsible for these clinical signs.

### PRINCIPLE

The primer and probe mix targets the specific conserved sequence encoding the *ssrRNA* gene. With the PCR reaction mix provided, the amplification of template can be quantitatively monitored by the increasing fluorescence signal detected by a real-time PCR instrument.

The PCR detection system includes an endogenous internal control primer and probe mix. The result of internal control provides the accuracy of sampling and extraction process, in order to avoid false-negative results.

**Canine Anaplasma spp. Real Time PCR Kit** contains amplification reagents, consisting of the following:

Description	Quantity	Storage
1. Test	10 Vials	RT
2. Positive Control	1 Vial	RT
3. Negative Control	1 Vial	RT
4. Resuspension Buffer	100 µl/vial	RT

### INSTRUMENT COMPATIBILITY

**Canine Anaplasma spp. Real Time PCR Kit** is compatible with the following:

Real-time PCR Instruments with channels
FAM
HEX
ROX

### OTHER MATERIALS APART FROM KIT COMPONENTS, REQUIRED TO PERFORM THE TEST.

New pair of disposable gloves and facemask  
Biohazardous waste container  
DNA extraction kit

### STORAGE AND STABILITY

- Shelf-life of components is 12months. Manufacture date is indicated on the box.
- Reagents should be stored in dark at Room Temperature.
- The reconstituted liquid reagent should be used up at once.

### SPECIMEN REQUIREMENT

- Sample Type: Whole blood, serum, Plasma, tissue of infected animals.
- Sample Collection: Collection accordance with conventional sample collection methods.
- Sample Storage and Transportation: Sample to be tested can be processed immediately or stored at  $-20^{\circ}\text{C}$  ( $\pm 5^{\circ}\text{C}$ ) for 3 months, or  $-70^{\circ}\text{C}$  for the long term. Avoid repeated thawing and freezing. Sample should be transported with refrigerant packs in a sealed Styrofoam box or ice chest.

### WARNINGS AND PRECAUTIONS

- This product is to be used only for *in vitro* diagnostic detection. For use only by laboratory-trained professionals. Please read this manual carefully before use.
- The contamination of laboratory environment and reagent, or cross-contamination during specimen treatment may lead to a false-positive result.
- Operation procedure and precautionary warnings of this instrument should be well understood before conducting the test. Quality control should be performed for each test.
- The decrease of detection effect: A false-negative result may occur if there are any mistakes in the transportation, storage and operation of reagents.
- Handle all specimens as if infectious, using safe laboratory procedures. All samples should be regarded as potentially infectious materials. Laboratory workers should wear disposable gloves, laboratory coat/gown, etc. Gloves should be changed after handling each sample, to avoid contamination and false results. Laboratory management should be strictly in accordance with the regulations of PCR gene amplification laboratories. Laboratory personnel must be professionally trained, and the experiment process should be strictly divided into sections/organized. All consumables should be properly sterilized and used only once. Instruments and equipment should be assigned to each stage of the experiment and alternative use of the same should be prohibited.
- Inappropriate sample collection, transfer, storage and operation may lead to inaccurate test results. DNA extraction should be carried out as soon as possible after sample collection to avoid degradation. If it cannot be carried out immediately, it should be stored in accordance with suitable specimen storage procedures. As this test involves the extraction of DNA and PCR amplification, please take care to avoid contamination of the amplification reaction mixture. Also, regular monitoring of laboratory contamination is recommended.
- When using this kit, please follow the instructions strictly. The collection, storage and transfer of samples, the extraction and detection of DNA, and the interpretation of results must be carried out in strict accordance with the requirements specified in the kit instructions. The processes of sample preparation and addition must be carried out in the biosafety cabinet or other basic protective facilities according to the technical requirements of the regulatory standards.
- The operation of sample and waste should meet the requirements of relevant laws and regulations. Discard all materials in a safe and acceptable manner, in compliance with all legal requirements. If exposure to skin or mucous membranes occurs, immediately wash the area with large amounts of water. Seek medical advice immediately. Do not use components beyond the expiration date printed on the kit boxes. Do not mix reagents from different lots. Return all components to the appropriate storage condition after preparing the working reagents. Do not interchange vial or bottle caps, as cross-contamination may occur. Reagents supplied are formulated specifically for use with this kit. Make no substitutions in order to ensure optimal performance of the kit. Further dilution of the reagents or alteration of incubation time and temperature may result in erroneous or discordant data.

### PREPARATION BEFORE TESTING

Please follow user manual instructions to extract DNA from clinical sample using a DNA extraction kit. Extracted DNA can be used directly for PCR detection. Otherwise, keep DNA sample at  $-70^{\circ}\text{C}$  if not in use. Avoid repeated thawing and freezing.

**Note: This product does not contain DNA extraction kit and is compatible with other commercial kits.**

### DETECTION METHOD

#### 1. TEST

Take out the test vial and add 20 µl of the extracted sample to resuspend the mix. Let it sit for 30 seconds, then gently pipette up and down to ensure thorough mixing. Spin the tubes for 30-30 seconds, then transfer the entire volume into a PCR strip or well for testing.

#### 2. POSITIVE AND NEGATIVE CONTROL

Add 20 µl of resuspension buffer and gently pipette up and down until the mix dissolved completely and spin the tubes for 30 seconds. Avoid generating air bubbles. Wash the wall of the tube by pipetting thoroughly. Aliquot completely to PCR strip or well.

**Note: The reconstituted liquid reagent should be used up at once.**

#### 3. SETTING UP PCR

- Place the tubes on the sample holder in the instrument. Set up the test panel according to the positions of positive control, negative control and test samples.
- Select the detection channels as following:  
Select FAM (Internal control) HEX (*A. platys*) and ROX (*A. phagocytophilum*) channels to detect Canine Anaplasma spp.

3.3. Enter the amplification program commended as below:

Step	Temperature	Time	Cycle
1 Incubation	50°C	2 minutes	1
2 Initial Denaturation	95°C	2 minutes	1
3 Denaturation	95°C	10 seconds	50
4 Annealing, Extension & Fluorescence measurement	60°C	20 seconds	
Cooling	25°C	10seconds	1

Save the file after making the settings and run the reaction. Please set the fluorescence internal control of the instrument to "None". For example, for ABI series instruments set "Passive Reference" to "None".

**4. RESULT INTERPRETATION:** Please refer to the user manual of the instrument for setting the following analysis uses ABI series instruments as an example.

4.1. After the reaction is completed, the results are automatically saved and the amplification curves of the detected target DNA and the internal control are analyzed separately.

4.2. According to the analysis, the amplification plot will adjust the Start value End value and Threshold value of the Baseline (users can adjust the values according to the actual situation. Start value can be set with in 3~15, and End value can be set within 5~20; users can adjust the amplification curve of negative control to make it linear or below the threshold line). Click "Analyze" to perform the analysis and the parameters should meet the requirements mentioned in "Section 5. Quality Control". Lastly, record the qualitative results in the Plate window.

**QUALITY CONTROL**

**Canine Anaplasma PCR Negative Control:**

A. plays (HEX) and A. phagocytophilum (ROX) channels does not show Ct value or Ct>35.

**Canine Anaplasma PCR Positive Control:**

Internal Control (FAM), HEX, ROX channels shows at Cts35

The above requirements must be met at the same time in the same experiment otherwise, this experiment is invalid and needs to be repeated.

**RESULT INTERPRETATION**

**Positive Threshold**

According to the study of the reference value, the Ct reference value for the target gene detected by this product is 35, and the Ct reference value of internal control is 35.

**Result Analysis**

Internal control (FAM)	A. plays (HEX)	A. phagocytophilum (ROX)	Conclusion	Remark
Cts35	Has amplification curve; Cts35	Has amplification curve; Cts35	Positive	Report results to the Sender
Cts35	No amplification curve	Has amplification curve; Cts35	-VE for A. plays and +VE for A. phagocytophilum	Report results to the Sender
Cts35	Has amplification curve; Cts35	No amplification curve	-VE for A. phagocytophilum and +VE for A. plays	Report results to the Sender
Ct>35	-	-	Invalid; need collecting sample again	Retest

1. First, analyze the amplification curve internal control FAM channel. If Cts35, it indicates that the detection is valid, and users can continue the subsequent analysis:

- a) If a typical S-type amplification curve is detected by the HEX/ ROX channel, with Cts35, it indicates that A. plays /A. phagocytophilum is positive.
- b) If HEX/ROX channel does not detect a typical S-type amplification curve (no Ct), it indicates that A. plays /A. phagocytophilum is negative.

2. If the internal control FAM channel failed to detect Ct or Ct>35, it indicates that the concentration of the tested sample is too low or there is an inhibitory reaction from the interfering substance. Users have to repeat the experiment.

3. For negative samples, the internal control should be positive. If the internal control is negative, the test result of the sample is invalid. The cause should be found and eliminated. Users should redo sampling and repeat the experiment (If the result is still invalid, please contact the manufacturer).

**LIMITATIONS**

1. The test results of this product are for clinical reference only.
2. Analysis of possibility of false-positive and negative results:
  - 2.1. Improper sample collection, processing and transportation and low sample concentration may cause false-negative results.
  - 2.2. Variations in the target sequence or sequence changes caused by other reasons may lead to false-negative results.
  - 2.3. Improper reagent storage can lead to false-negative results.
  - 2.4. Other unproven interferences or PCR inhibitors may cause false-positive results.
  - 2.5. Delayed sample processing, may cause false-positive results.
  - 2.6. This assay should be performed according to Good Laboratory Practice (GLP) regulation. Operators should strictly follow the manufacturer's instructions in performing the test.

**PRODUCT PERFORMANCE**

**Specificity**

The primer and probe provided is designed based on the conserved sequence of the Canine Anaplasma srrRNA gene and has high detection rate of the target gene fragment. The specificity of the kit was checked and this product has no cross-reactions among positive samples of CCV, CDV, CPV, Canine herpes virus, Canine adeno virus 1&2, Canine para influenza, Babesia. The negative and positive rates of detecting commercial reference materials were 100%. The observed values for the Sensitivity of the test kit were 95%.

**Linear Range**

For the determination of the linear range of the Canine Anaplasma Real Time PCR Kit, a dilution series Canine Anaplasma DNA ranging from 100000 - 20 IU/ml prepared in replicates.

The linear range of the Canine Anaplasma Real Time PCR Kit for the quantification of Canine Anaplasma is 40 - 200000 IU/ml.

**DATE OF REVISION**

09.04.2025

To report any adverse events, write to [contact@thegenes4life.com](mailto:contact@thegenes4life.com) or call us on +91 9778698070



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