FLASHTEST

【Product Name】
Parrot sex determination(P-Sex.d) Nucleic Acid Test Kit (Lyophilized)

【Package Specifications】
4 T/box

【Intended Use】
This kit uses fluorescence PCR methods to detect the nucleic acid of parrot gender in the samples (P-Sex.d).
This product requires operation with a real time quantitative PCR instrument and can achieve rapid POCT detection.

【Testing Principle】
The test kit uses nucleic acid extraction reagents to extract the nucleic acid (DNA/RNA) from the sample.
Under the action of a high-efficiency reverse transcriptase, cDNA complementary to the RNA template is synthesized in a one-step reaction using RNA as the template.
Under the action of Taq enzyme, the copy number of the specific target fragment is amplified through cycles of high-temperature denaturation, annealing at a moderate temperature, and extension using DNA as the template.
The fluorescence-labeled specific probe hybridizes with the amplified target fragment, and the 5'→3' exonuclease activity of Taq polymerase separates the reporting group and quencher group of the fluorescence probe, emitting a specific fluorescence signal.
The specific fluorescence signal is detected using a fluorescence PCR instrument, and the result is determined based on the Ct value of the sample and the formation of the amplification curve.

【Contents】

Item	Quantity	Storage
PCR master mix	4 pcs	-20℃ (Away from light)
Instructions for use	1 pcs	Room Temperature
Sample buffer	4 pcs	
Swab	4 pcs	
Biohazard bag	4 pcs	

【Storage conditions and shelf life】
1. Shelf life: 24 months.
2. Production date and expiration date are on the package.

【Compatible Instruments】
This test kit is compatible with FLASHTEST real-time quantitative fluorescence PCR instrument.

【Sample】
Fingertip blood, feather

【Sample Handling】
1. Fingertip blood: Collect blood with a swab. With the swab in the sample buffer, shake it thoroughly to fully dissolve the pathogen on the swab head into the buffer.
2. Feather: Take 1 contour feather (cut 1 to 2 cm from the root) or take 3 to 5 down feathers, grind them in the sample buffer, centrifuge for 1 minute, collect the supernatant.
3. Add 200 μL of mixed buffer to the nucleic acid extraction cartridge for extraction.

【Specimen storage】
Samples used for nucleic acid extraction and detection should be tested as soon as possible.
Samples to be tested within 24 hours can be stored at 4°C.
Samples that can not be tested within 24 hours should be stored at -20°C for up to 10 days.
Avoid repeated freezing and thawing of samples.

【Instructions for Use】
1. Add Elution
1.1 Add 20μL of elution from magnetic bead extraction, to each PCR tube. Close the lid tightly.
1.2 Shake all the liquid to the bottom of the PCR tube. Use the vortex mixer to mix the PCR tube thoroughly, for 5 seconds. After mixing, make sure all liquid is at the bottom of the PCR tube, by shaking the tube again. (optional: use a small centrifuge for 3 seconds to shift all liquids to the bottom.)

2. PCR Amplification
2.1 Set the parameters as follows:

Step	Temperature	Time	Cycle
1	55℃	3min	1
2	95℃ 59℃	5s 20s	×40

2.2 The reaction volume is 20μL. Fluorescence channels:

Channel	FAM	VIC	CY5	ROX
Detection Item	W gene	Z gene		

3. Result Interpretation
3.1 Reference Range

Parameter	Reference Range	Result Interpretation
Z gene	Ct ≤ 37 and there is a clear exponential amplification curve	Valid
	Ct > 37 or No Ct	Invalid
W gene	Ct ≤ 37 and there is a clear exponential amplification curve	Female
	Ct > 37 or No Ct	Male

3.2 Test Result Interpretation

W gene Result	Z genel Result	Test Result Interpretation
Female	Valid	Female
Male	Valid	Male
Any Result	Invalid	Test invalid, please retest

【Test Limitations】
1. The test results of this kit should be comprehensively analyzed in conjunction with other relevant physical examination results and should not be used as the sole basis for diagnosis.
2. Improper sample collection, transportation, storage, handling, and inadequate laboratory conditions may lead to inaccurate results.
3. Other unconfirmed interferences or PCR inhibitors may lead to false negative results.
4. Sequence variations caused by mutations or other factors in the target gene of the virus being tested may lead to false negative results.

【Product Performance】
1. Positive and negative control consistency: The positive and negative controls included in this test kit have been tested with the company's working reference materials, and the positive and negative compliance rates are both 100%.
2. Sensitivity: limit of detection is 500 copies/mL.
3. Specificity: This assay does not cross-react with non-target pathogen samples.
4. Precision: The coefficient of variation (CV, %) of the Ct values for 10 consecutive tests of one strong positive sample and one weak positive sample is ≤5%.

【Notes】
1. Before using a PCR kit, check the lyophilized PCR mix at the bottom of the tube is in good condition (white and clumped). Liquified lyophilized PCR mix can not be used. After opening, it should be used as soon as possible or stored away from light.
2. This product is only for in vitro testing (for animals). All operations must strictly follow the instructions.
3. Overloading samples may result in false negatives. Retest is recommended.
4. Avoid bubbles in PCR tubes. Keep the tube cap firmly closed.
5. Use disposable tips, gloves, and laboratory coats.
6. After tests, disinfect the workbench with 10% hypochlorous acid, 75% ethanol, or UV light.
7. All items in the kit should be treated as biowaste and handled in accordance with local laboratory regulations.