SUNLONG
SunLong Biotech Co.,LTD
Tel: 0086-571- 56623320 Fax:0086-571- 56623318
E-mail:sales@sunlongbiotech.com
www.sunlongbiotech.com

General PCR Detection Kit for *Hemotropic mycoplasma*

Mycoplasma haemophilum (Hemotropic mycoplasma, or hemoplasma) is a type of prokaryote that parasitizes the blood of various mammals, attaching to the surface of red blood cells. It lacks a cell wall and exhibits pleomorphism, capable of spreading through the bloodstream. M. haemophilum was formerly named Haemophilus (Eperythrozoon) and Bartonella (or Bartonella) (Haemobartonella), classified as Rickettsia. Based on 16S rRNA gene sequence analysis, it has since been reclassified as Mycoplasma. Clinically, M. haemoph- ilum can cause hemolytic anemia, with other symptoms including anorexia, somnolence, dehydration, weight loss, and sudden death. Sometimes it presents as an acute onset, while many animals show mild or no symptoms after infection. M. haemophilum has been found in cats, dogs, pigs, cattle, sheep, camels, and rodents, with many animal blood infections still being unknown. It is generally believed that hematophagous arthropods are its transmission vectors.

The in vitro cultivation of Mycoplasma hyoransa is extremely challenging and not commonly applied. The detection method generally uses the Gram stain blood smear technique. This method has poor sensitivity and specificity, and is easily affected by the preparation method and other components in the blood. In contrast, using PCR for detection is more convenient, with higher sensitivity and shorter testing time, yielding results within just a few hours.

This kit is designed for PCR identification of the 16S rRNA gene, with primers specifically targeting Mycoplasma haemolytica. It can identify all known strains of M. haemolytica parasitizing common mammals (such as cats, dogs, pigs, cattle, sheep, camels, and rodents), as shown in the table below; BLAST validation has confirmed that it does not cross-react with eukaryotic cell genomes but only with non-haemolytic Mycoplasma species of the Mycoplasma fastidiosum group, including: Mycoplasma lionii (Candidatus Mycoplasma liparidae) (discovered in the gut of deep-sea lionfish), M. Mycoplasma fastidiosum (discovered in the nasopharynx of horses), M. Mycoplasma cavipharyngis (discovered in the nasopharynx of guinea pigs), and M. Mycoplasma insons (discovered in the upper respiratory tract of green iguanas). For the wild boar (wild boar) strain of M. pig (Mycoplasma suis), this kit has lower sensitivity. Testing 100 cat blood DNA samples yielded 22 positive results, which were reconfirmed using species-specific PCR, identif- ying one or several of the three types of feline haemolytic Mycoplasma strains, with no false positives. This kit is suitable for preliminary detection and screening of haemolytic Mycoplasma in samples.

Mycoplasma	**Host**	**Mycoplasma**	**Host**
Mycoplasma coccoides Candidatus Mycoplasmahaemomuris	Mouse Mouse	Mycoplasma ovis Candidatus Mycoplasmahaemovis	Sheep Sheep
Mycoplasma haemofelis Candidatus Mycoplasma turicensis Candidatus Mycoplasma haemominutum	Cat Cat Cat	Candidatus Mycoplasma erythrocervae Candidatus Mycoplasma haemocervae	Deer Deer
		Candidatus Mycoplasma kahanei Candidatus Mycoplasma haemomacaque	Monkey Monkey
Mycoplasma haemocanis Candidatus Mycoplasma haematoparvum	Dog Dog		
Mycoplasma suis Mycoplasma parvum	Hog Hog	Mycoplasma erythrodidelphis	Didelphis lanigera
Mycoplasma wenyonii Candidatus Mycoplasma haemobos	Cattle Cattle	Mycoplasma haemozalophi	Sea lion
Candidatus Mycoplasma haemohominis	Human	Candidatus Mycoplasma haemomeles	Meles meles
		Candidatus Mycoplasma haemolamae	Alpaca

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Part number: PCR-Mhemo-20 **Specifications: 20 reactions**
Part number: PCR-Mhemo-50 **Specifications: 50 reactions**
Part number: PCR-Mhemo-100 **Specifications: 100, reactions**

Reagent kit components:

Builder	Element	Volume (microlitres)		
		PCR-Mhemo-20	PCR-Mhemo-50	PCR-Mhemo-100
Group 1A (blue tube)	Taq enzyme,dNTPs,dye	250	625	1250
Group B (green tube)	Upstream and downstream primers	50	125	250
Component group C (yellow tube)	Positive control samples	50	125	250
Water (white tube)	Sterile ultrapure water	200	500	1000

Storage conditions: Store at-20 , avoid repeated freezing and thawing.
Without repeated freezing and thawing, the shelf life is one year.

Method of operation:

1. Sample preparation

Prepare EDTA-treated blood and extract sample DNA using a DNA extraction kit for use in the PCR reaction.

2、 Preparation of the PCR reaction

After the components of the reagent kit have melted, perform an immediate centrifug- ation, then gently tap to mix. Refer to the table below for preparing the reaction system in PCR tubes. Each experiment requires one negative and one positive control, and multiple test reaction tubes can be used. During preparation, pay attention to aerosol contamination and avoid vigorous operations. After preparation, perform an immediate centrifugation to allow the liquid to settle at the bottom of the tube. Use large-volume pipettes as much as possible; the larger the volume, the smaller the pipetting error.

Volume (microlitres)	Negative control tube	Positive control tube	Test reaction tube*
Builder A	12.5	12.5	12.5
Builder B	2.5	2.5	2.5
Builder C	–	2.5	2.5 / - *
Water	10	7.5	5.5 / 8 *
Test samples	-	-	2
Bulk volume	25	25	25

A ding component C to the test reaction tube can determine whether the test sample contains PCR inhibitors. When component C is added to the test reaction tube and reacts with the sample, if a band (1108bp and/or 595-622bp) appears, it indicates no PCR inhibitors. If no band appears, it suggests the presence of PCR inhibitors. In this case, the possibility of false negatives should be noted.

3、 PCR reaction

Preheat at 94℃ for 5 minutes; denature at 94℃ for 20 seconds, anneal at 60℃ for 30 seconds, extend at 72℃ for 70 seconds, and cycle 30 times; after the cycle is over, extend at 72℃ for 7 minutes, and finally maintain at 4℃.

4. Agarose gel electrophoresis

Prepare a 1.5% agarose gel using conventional methods and add EB or Gold-View for color development. Take 8 microliters of each reaction mixture, without adding loading buffer, and directly add it to the well for gel loading. Leave another well for DNA marker (preferably showing bands in the 600-1100bp range), and run the electrophoresis at 120 V for about 30 minutes. Observe the electrophoresis results under UV light; the positive control should show a band at 1108 bp, the negative control should show no band, and the Mycoplasma hyorad decans should show bands in the 595-622bp range.

Matters need attention:

1. Component A contains dye, the addition of dye does not affect the PCR reaction, and the reaction products can be directly electrophoresed, saving time.

2. PCR is highly sensitive; even trace amounts of aerosol generated during operation can cause cross-contamination between samples. Therefore, extreme caution must be exercised to avoid vigorous handling. When adding reagents, the pipette tip should ideally be pressed against the wall of the tube. All tubes should be capped immediately after use. Control and test samples should be added last. Pipette tips used for sample handling should be discarded immediately after use to minimize the risk of contamination during operation. Use non-polluting disposable pipette tips, preferably those with filter cartridges, and operate in a well-ventilated clean area. If this product is used long-term, always use pipette tips with filter cartridges and take care to avoid environmental DNA contamination on the tips. Wear dust-free gloves during operation.

3. It is best to divide the work area into different areas for different steps, such as DNA extraction and PCR reaction preparation, at different stages.

4.This product is for scientific research only and not for diagnostic purposes.