

Rabies Virus Identification Multiplex Detection Kit

(Field isolated RV & Vaccine strains)

One-step RT-PCR Detection Kit
for Field isolate RV & Vaccine strains



DESCRIPTION

Rabies is the most devastating zoonosis in the world. Every year, 25,000 to 75,000 people are estimated to be infected by rabies and among them, more than 29,000 cases occur in China and Asian region. Rabies usually spread out through the animals such as bat, red fox, skunk, raccoon, dog, wolf and mongoose. In the case of Korea, dog mediated transmission has been vanished and only mongoose or badger mediated transmission is remained since 1993. The pathogen of rabies is rabies virus. People generally get infected when they are bitten by cats or dogs, however any kind of warm-blooded animals can be a carrier of rabies virus. Also, there has been a report for bat rabies which shows a similar symptom with the paralytic rabies (dumb rabies). The morbidity of rabies is ranged from 5 to 50% and incubation period is related with the amount of virus, depth of the wound, sparseness of neural codes in wound site, affected area. After 10-100 days from onset of rabies, it is impossible to treat and mortality becomes 100%. The time to see the symptom in every infected subjects are affected by several reasons and vary from one week to one year. In general, rabies occurs 1-2 months later from initial exposure to rabies virus and if the wound is closer from the brain the faster to show the symptom. In the early stage of rabies, indistinctive symptoms such as pyrexia, headache, lethargy, losing appetite, nausea or a dry cough occur for 1-4 days. If there is any discomfort or a pricking or itching sensation at the site of bite, it can be suspected as the symptom of rabies infection. Since the vaccination against rabies virus has been generalized, there is a growing need for discriminating vaccine strain from wild strain and that is why we developed Rabies Virus Identification Multiplex Detection Kit(Field isolated RV & Vaccine strains). Rabies Virus Identification Multiplex Detection Kit(Field isolated RV & Vaccine strains) is designed to be able to distinguish field strain from ERAGS vaccine strain. Furthermore, highly efficient HiSenScript RTase and i-StarTaq GH DNA Polymerase are used to make the Kits highly sensitive and specific. Plus, the Kit is in a ready-to-use form, means all components are pre-mixed so users only need to add template RNA and DW to proceed RT-PCR.

CAUTIONS



1. All chemicals are potentially dangerous. And the test samples are possible to be infectious reagents in human body.
2. Always wear protective gear during handling chemical materials and the test should be handled by professionally trained person.
3. Be careful and prevent the contamination and direct contact from the test samples.



1. The test samples are handled under the condition of unknown level (concentration), so the laboratory contamination is expected. Therefore, all glasses used for experiments must be sterilized and secure the personal safety.
2. Separate the place where handling samples, extracting genes, amplification and etc. and be careful for the contamination that may exist in the air and surface.

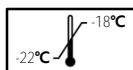


1. This Kit **can't** be used for definite diagnosis.
2. If there is too much DNA, several band may be shown. In that case, the amount of DNA should be decreased.
3. All the PCR product should be discarded after UV irradiation (10 min/365nm) to prevent carry-over contamination.
4. All the waste should be sterilized before discarding.
5. DNA extraction should be carried out using DNA extraction Kit.
6. The test samples should always be handled on the Biological Safety Cabinet until the sample is dissolved completely.
7. Store samples at -20°C.

KIT CONTENTS

- RV Identification Multiplex premix strip (8-tubes) 48 tubes (6 strips)
- ERAGS Positive Control (yellow cap) 25 µl x 1 vial
- Other Positive Control (orange cap) 25 µl x 1 vial
- DNase-RNase free water (white cap) 1 ml x 1 vial

STORAGE



Store the product at -22 ~ -18°C after receiving. Under these conditions, The Kit can be stored for up to 12 months. The expiration date is indicated on the product box.

ADDITIONAL REQUIRED MATERIALS

- Real time PCR Instrument
- Electrophoresis apparatus, UV transilluminator
- Disposable gloves
- Viral RNA extraction kit
- Pipettes
- Sterile pipette tip (with filter)
- Vortex mixer
- Centrifuge for micro-centrifuge tubes



Distribuito in ITALIA da
Li StarFish S.r.l.
Via Cavour, 35
20063 Cernusco S/N (MI)
telefono 02-92150794
fax 02-92157285
info@listarfish.it
www.listarfish.it

PROTOCOL

1. Wipe the workspace and the surface of pipette with diluted household bleach solution using Kimwipes.
2. Prepare the RV Identification Multiplex premix strip then label the each tube.
3. Add 2 µl of template RNA into the RT-PCR premix tube.
4. Add 18 µl of DNase/RNase-free water into the RT-PCR premix tube to total volume as 20 µl.

Contents	Volume
1 Template DNA	2 µl / tube
2 DNase-RNase free water	18 µl / tube
Total volume (/ tube)	20 µl

5. Mix the mixture well, then centrifuge the tube to gather residual solutions.
6. Follow the procedure as indicated in the Table below.

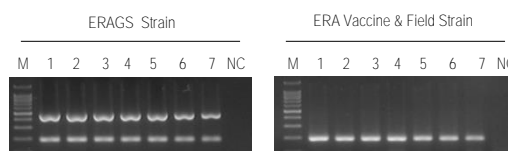
RT-PCR condition			Cycle
Reverse transcription reaction	45°C	30 min	1 cycle
Inactivation of Reverse transcriptase	94°C	5 min	
Denaturation	94°C	30 sec	40 cycles
Annealing	55°C	30 sec	
Extension	72°C	30 sec	
Final extension	72°C	5 min	1 cycle

7. Load 7 µl of PCR product on agarose gel (1.5% agarose gel with RedSafe™ Nucleic Acid Staining Solution (20,000x) or EtBr) then run electrophoresis at 110V for 40 minutes. Identify the result on ultra-violet (UV) transilluminator.

INTERPRETATION

Expected PCR product size is indicated below.

Target	Target size
ERAGS Strain	459 bp / 192 bp
ERA Vaccine & Field Strain	192 bp



PREVENT CROSS CONTAMINATION

Each PCR/RT-PCR Pre-mixture contains 8-methoxypsoralen (8-MOP) to prevent carry-over contamination. When the 8-MOP is irradiated under UV light for 20-30 minutes, it activated then starts to disturb the PCR products to react with template DNA.