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Ref. SB0021

MANUAL

Expiry date: 1 year

STORE AT -20°C

GENEKAM ONE STEP RT-MASTER MIX

**-Only for research use-
-To be used by a technical person-**

Genekam RT-master mix can be used with RNA isolated from blood, tissue or any other source. It is successfully tested for use in detecting the RNA viruses like all kind of influenza viruses, Parainfluenza viruses as well as Bovine viral diarrhoea virus (BVDV).

This PCR-Mastermix can be used for realtime as well as conventional PCR. It can be used with fluorogenic probes. Fluorogenic probes are also known as Taqman probes.

Contents of shipment: tube B and tube Y.

Contents: DNA polymerase, reverse transcriptase, nucleotides, MgCl₂ and other chemicals.

How to use this: it is sufficient to have total volume of 20 ul/reaction: 10 ul of Genekam tube B + 1ul of tube Y + 0.2-1 uM primers + 1-3ul RNA + molecular grade water (to fill the rest volume : the molecular grade water should be of good quality).

Temperature to be added before your own test-specific temperature:

1. 3000 seconds at 42°C
2. 600 seconds at 48°C
3. 600 seconds at 70°C
4. 180 seconds at 94°C
5. your own testspecific temperature

In case of nested PCR, we recommend to use 5 ul of Genekam DNA Master Mix (SB001/12) or other Master Mix in last step, however you have to see what is the optimal amount to run the nested PCR as it may be possible that you may need between 2.5 ul-10 ul master mix. In single step PCR, in some reactions 5ul may be sufficient as you have to test yourselves.

Here is one example to do PCR test for the human influenza virus : 10 ul of Genekam tube A + 1ul of tube B + 1-2 ul of 1-2 pmol of primers (some times, you may need more; test yourselves) + 1-3ul of RNA + rest should be filled with molecular water (total volume 20ul).

For optimal specificity and amplification, one needs to do individual optimization.

This can be used in gel agarose PCR as well as real time PCR.

Recommendations: One should avoid frequently thawing. Therefore one can make the aliquots and put one aliquots at 4 degree for 2-3 weeks.

If you should find any mistakes, please let us know. Thank you.

Suggestion:

This manual has been written specifically for beginners, hence persons with experience in PCR must use their experience to keep each step as small as possible e.g. you should calculate the amount of the needed chemicals, before starting with testing.

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Genekam Biotechnology AG

Dammstr. 31-33
47119 Duisburg
Germany
Tel. (+49) 203 / 555858-31,-32,-33
Fax (+49) 203 / 35 82 99
anfrage@genekam.de
<http://www.genekam.de>