Lot.

MANUAL

Ref. SB0022

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 RNA 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 diarrhae virus (BVDV).

contents: DNA polymerase (1 unit/ul) , reverse trancriptase , dATP, dCTP, dGTP, dTTP, MgCl2 and other chemicals.

How to use this: it is sufficient to have total volume of 20 ul: 10 ul of Genekam RNA Mastermix + 0.2-1 uM primers + 1-2 ul RNA + molecular grade water (to fill the rest volume : the molecular grade water should be of good quality).

In case of nested PCR, we recommend to use 5 ul of Genekam DNA Master Mix 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 RNA Master Mix + 1-2 ul of 1-2 pmol of primers (some times, you may need more; test yourselves) + 1-2 ul of RNA + rest should be filled with molecular water .

After this, please run the PCR reaction in the PCR machine according your protocol, but the following temperature must be added before adding your program. It is 59 degree for 2700 second and 95 degree for 600 seconds in order to convert the RNA in cDNA. After this, kindly add your programm. It can be done at the same like the following example:A) 59 degree for 2700 seconds and 95 degree for 600 seconds B) 30 cycles of 94 degree for 30 seconds, 60 degree of 45 seconds, 72 degree of 30 seconds. In case you are not sure, kindly send us a mail to be sure (query@genekam.de).

For optimal specifity and amplification, one needs to do indivual 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.