



Certificate of Analysis



Technical Data

Non infectious reagent, non replicating

Product name: MERS-CoV upstream E (upE) assay (screening assay)

Virus: Betacoronavirus MERS-CoV (hCoV-EMC)

Nucleic acid type: (in-vitro) transcribed RNA

Quantity: 1 x 100 µl, sufficient for 2000 reactions

IVT stock concentration: 1,0E+5 copies / µl

Reference: : Corman et. al; Eurosurveillance, Volume 17, Issue 39, 27 September 2012

Usage: serial dilute product 1:10; 1:100; 1:1,000; 1:10,000 upon arrival and store at - 80°C

Use the 1:100, 1:1,000 and 1:10,000 dilution in the assay.

<u>Dilution</u>	<u>RNA Copies / 5 µl IVT</u>
1:100	5,000
1:1000	500
1:10000	50

Mastermix:

2.6 µl H₂O (RNase free)
0.4 µl MgSO₄ (50mM)
12.5 µl 2x Reaktions-Mix*
1.0 µl BSA (1 mg/ml), Roche**
1.0 µl Fwd Primer (10µM)
1.0 µl Rev Primer (10µM)
0.5 µl Probe (10µM)
1.0 µl SSCIII/Taq EnzymeMix*

20 µl + 5 µl RNA template

Reagents

*Invitrogen SuperScript III OneStep RT-PCR System mit Platinum Taq, #12574-026

**Roche BSA (20 mg/ml), #10711454001

Non-acetylated. This component ist only necessary if using glass capillary LightCycler. Can be replaced with water in plastic vessel machines such as ABI 7500, LC480, etc.

LightCycler. cycling conditions

55°C	20 min
94°C	3 min*
45 cycles	
94°C	15 sec
58°C	30 sec. single read step. F1(530nm)

* Activation time may vary according to used enzyme

Primer/Probe sequences

upE-Fwd GCAACGCGCGATTTCAGTT
upE-Prb FAM-CTCTTCACATAATCGCCCCGAGCTCG-TAMRA
upE-Rev GCCTCTACACGGGACCCATA