enderMASS

The ender MASS is an in-vitro diagnostic test kit based on a rapid molecular isothermal nucleic acid amplification technology [1] and a greatly simplified sample preparation procedure as compared to ender LAB. It is intended for the qualitative detection of nucleic acid ORF-1a gene sequence from the SARS-CoV-2 viral RNA [2] in direct nasal, nasopharyngeal or throat swabs from individuals who are suspected of COVID-19 by their healthcare provider. The test is performed on standard Real-Time Polymerase Chain Reaction (RT-PCR) cyclers (further specification details see below).

The ender MASS test kit identifies SARS-CoV-2 RNA in clinical samples. The SARS-CoV-2 RNA is generally detectable in respiratory samples during the acute phase of infection [3]. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results should be considered in the context of a patient's recent exposures, history and the presence of clinical signs and symptoms consistent with COVID-19.

The ender MASS test kit is intended for use by medical professionals or trained operators who are proficient in using RT-PCR cyclers and standard molecular biological laboratory procedures.

Test procedure principles:

ender MASS is based on an isothermal amplification [1] and real-time detection technology of the SARS-CoV-2 ORF-1a gene sequence [2]. The amplification reaction takes place at a constant temperature of 65 °C. No thermal cycling is required since the polymerase harbours a helicase activity. Thus, primer binding and amplification happen instantaneously and continuously. A separate reverse transcription step is not required due to reverse transcription activity of the enzyme used. Reverse transcribed and amplified DNA is detected by a fluorescent DNA-intercalating dye. After isothermal amplification a melting curve analysis is performed to distinguish the SARS-CoV-2 amplicon from the included internal amplification control (IC). The two targets are identified based on their melting temperature. The required total reaction time is 30 minutes.

Sample preparation does not require time-consuming RNA extraction. Instead, a simple heating and dilution step is performed to prepare the sample.

Reagents and materials:

Color of cap / Symbol	ender MASS-80 (Cat No: EM16S)	ender MASS-240 (Cat No: EM16M)	ender MASS-400 (Cat No: EM16L)
total number of reactions	80	240	400
Blue / 1	2x 160 µl	6x 160 µl	10x 160 µl
Green / 2	2x 1200 µl	6x 1200 µl	10x 1200 µl
Yellow/ lys	2x 1600 µl	6x 1600 µl	10x 1600 µl
Red / pos	1x 40 µl for 5 runs	2x 40 µl for 10 runs	2x 40 µl for 10 runs

Tube "1" (blue lid): Primer mix including primers for SARS-CoV-2, the internal control (IC) and the target sequence of the IC

Tube "2" (green lid): Amplification enzyme in reaction buffer

Tube "lys" (yellow lid): Lysis buffer (non-hazardous)

Tube "pos" (red lid): positive control (non-infectious, non-hazardous plasmid)

Negative control: use H_2O of molecular grade (not provided with the test kit).

Specimen collection:

Use swab specimen collection kits released for sample collection in the upper respiratory tract using UTM or Amies buffer for storage and transport of samples until processing. Transport media containing Guanidine-thiocyanate will inhibit the amplification reaction and can not be used. Follow the instructions for use provided by the manufacturer of the specimen collection kit.

Sample preparation procedure:

We recommend aliquoting the lysis buffer into 40 µl aliquots (steps 1. and 2.) upon arrival of the kit.

1. Mix the lysis buffer (tube «lys» with yellow lid) well by vortexing
2. For each sample to be analyzed, pipet 40 µl of the lysis buffer into a 1.5 ml reaction tube. Use a pipet tip with a big opening (e.g. 1000 µl pipet tip). Important: to have all components of the lysis buffer in solution, vortex the «lys» tube quickly after every 5 samples
3. Add 100 µl of the sample
4. Heat for 2 min at 95°C in a thermoshaker, shaking at full speed
5. Immediately place the sample on ice and quickly add the sample to the prepared master mix (see below)

Use of ender MASS test kit:

Use reaction disposables as recommended by the RT-PCR machine manufacturer.

It is recommended to use RNase and DNase free plasticware and work on ice for the reaction set-up.

Procedure per one specimen and reaction: prepare the reaction mix in the following order:

1. add 30 µl of enzyme (tube 2 with green lid)
2. add 8 µl H_2O of molecular grade
3. Pipet 4 µl of primer mix including internal control (tube 1 with blue lid)
4. add 8 µl of the prepared sample of a specific specimen

Treat each specimen in a separate reaction.

Note: calculate the total amount of each reagent needed including all samples and controls and assemble a primer and enzyme master mix of 1), 2) and 3). Mix by vortexing carefully and briefly spin down. Aliquot 42 µl of the master mix into each reaction vessel and add 8 µl of the prepared sample or controls, each with a fresh pipette tip.

Measure a positive and negative control in each collective run on the RT-PCR cycler by using 8 µl of the positive control solution and H_2O of molecular grade instead of the prepared sample solution, but including all other reagents as outlined in 1), 2) and 3) above.

Following preparation of all reactions, place the reactions in the RT-PCR machine.

Amplification and identification of SARS-CoV-2 RNA ORF-1a gene sequence in non-extracted samples:

Use a standard RT-PCR machine, which allows to be programmed according to the amplification and detection protocol shown below.

Amplification method:

Isothermal amplification and melting curve analysis according to the following program:

1. 30 minutes at 65 °C, collecting fluorescence data once per minute
2. apply a temperature gradient from 80 °C to 90 °C for assessment of the melting temperature of the amplification product, continuously recording the fluorescence

Detection of results:

The fluorescence signal of SYBR Green / FAM or an equivalent channel (excitation at around 470 nm, detection at around 514 nm) is recorded for each reaction during the amplification and the melting curve step. Analysis occurs after the run.

Interpretation of results:

To determine if a sample contains SARS-CoV-2 RNA, compare the melting temperature (Tm) to the Positive and Negative Assay Controls. Ct values of the amplification curves are not relevant for the discrimination between a positive and a negative result. A positive, sigmoid amplification curve indicates the correct amplification of either the SARS-CoV-2 target sequence or the internal control (IC). If no sigmoid amplification curve is present the test has to be considered invalid due to inhibition or false handling. In this case, the sample has to be re-tested.

If the Tm of the sample corresponds to the Tm of the Negative Assay Control ±0.5 °C, it is considered SARS-CoV-2 RNA negative. In this case, the Internal Control was amplified. On the Roche LightCycler 96, the Tm of the IC is around 83.0 °C, on your instrument the Tm might be slightly different, please take down that value for reference to your samples.

If the Tm of the sample is between -0.5°C and +1.0 °C of the positive assay control, the sample is considered SARS-CoV-2-positive. On the Roche LightCycler 96, the Tm of SARS-CoV-2 is around 86.0 °C, on your instrument the Tm might be slightly different.

Samples with a low amount of SARS-CoV-2 RNA may show two melting peaks, one for the SARS-CoV-2 RNA target and one for the Internal Control. This result still has to be considered SARS-CoV-2 positive.

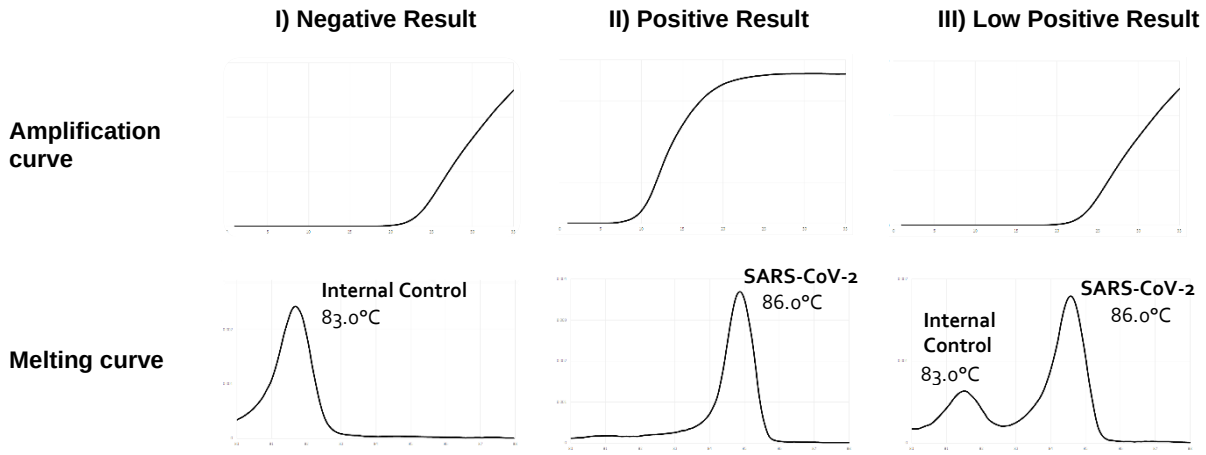

Figure 1: Test result interpretation

I. Validity of entire run is indicated by a negative result for the negative control (Tm 83.0 °C ±0.5 °C) and a positive result (Tm 85.5 °C ±0.5 °C) for the positive control.

II. Invalidity of entire run is indicated by a positive result (Tm 85.5 °C ±0.5 °C) for the negative control (contamination is suspected) and/or a negative result (Tm 83.0 °C ±0.5 °C) for the positive control. In case of invalidity of entire run, do not use results for any of the samples in the same run.

III. Internal control result in case of valid test procedure for samples: Positive result with a melting temperature peak at 86.0 °C ±0.5 °C (the amplification of the internal control is suppressed by the amplification of the sample SARS-CoV-2 target sequence). Result for corresponding sample is valid and can be used. The result for the sample is positive for the presence of SARS-CoV-2 ORF-1a gene sequence if a peak at a melting temperature of 86.0 °C ±0.5 °C is detected. The result for the sample is negative if a peak at a melting temperature of 86.0 °C ±0.5 °C is missing and a peak at a melting temperature of 83.0 °C ±0.5 °C for the internal control is present.

IV. Internal control result in case of invalid test procedure for sample: no result for the amplification curve detected with a missing peak for the melting temperature. In case of invalid test procedure as indicated by missing result for internal control, do not use the results for the corresponding sample.

Usage of test kit:

- The test kit is for in-vitro diagnostic use only
- Reliability of the results depends on adequate specimen collection, storage, transport and processing procedure
- The test kit reagents are single use only
- The workflow in the laboratory should proceed in a unidirectional manner with supplies and equipment dedicated to the specific working areas
- If not all reactions comprised in one test kit tube are used at a specific time, the residual reagents can be used consecutively. The user needs to make sure to apply good laboratory practice and ideally use filter tips not to contaminate any reagents by SARS-CoV-2 positive patient samples, positive control or amplicons.
- If test kits are used consecutively, the test kit must be stored in its original packaging under the indicated conditions.

Warnings:

I. Do not open the reaction tube after amplification to avoid potential contamination of the working area with amplicon potentially causing false results for other specimens.

II. Use only the reaction tubes recommended by the RT-PCR machine manufacturer for avoidance of potential opening of reaction tubes during amplification and detection procedure due to development of too high pressure in tube. Consequence would be potential contamination of working area with amplicon causing false positive results for other specimens.

III. Do not use the reaction for a second specimen, since the result will be always the one for the first specimen run.

IV. Do use the volumes per reaction as outlined in this IFU. Lower volumes lead to unreliable test kit performance and therefore to invalid results.

V. Do not mix ender MASS reagents with lab made reagents. Such procedure leads to unreliable test kit performance and therefore invalid results.

VI. Do not mix ender MASS reagents of different lots as indicated on the individual reagent tubes. Such procedure leads to unreliable test kit performance and therefore invalid results.

VII. Discard the closed reaction tubes according to your local regulation for non-infectious and non-hazardous specimens.

VIII. If spilled reagents or damaged tubes can be identified when opening the test kit packaging, the test kit must be discarded and cannot be used.

IX. ender MASS is a very sensitive assay and any contamination of the work area with target RNA may potentially lead to false positive test results. Therefore, samples need to be handled with high precaution and according to Good Laboratory Practice.

Limitations:

I. The performance of the ender MASS test was evaluated using the procedures provided in this product insert only. Modifications to these procedures may alter the performance of the test.

II. False negative results may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if amplification inhibitors are present in the specimen or if inadequate levels of viruses are present in the specimen. Negative results should be considered in the context of a patient's recent exposures, history and the presence of clinical signs and symptoms consistent with COVID-19.

III. As with any molecular test, mutations within the target regions of the ender MASS test could affect primer binding resulting in failure to detect the presence of the virus.

IV. The test cannot rule out diseases caused by other bacterial or viral pathogens.

Performance data:

The performance of ender MASS was evaluated using 74 nasopharyngeal swab specimens obtained from individuals with signs and symptoms of respiratory illness. The positive samples were checked by rtPCR as reference method and also confirmed to be positive by applying the protocol published by the WHO [4].

Diagnostic sensitivity: 93.0% (40/43 – 95% CI: 80.9%-98.5)

Diagnostic specificity: 100% (31/31 – 95% CI 88.8%-100%).

PPV: 100%,

NPV 99.2% (CI 97.7%-99.7%) at an estimated prevalence of 10%.

Analytical performance data:

Limit of Detection (LoD): 480 copies SARS-CoV-2 RNA / Reaction (AccuPlex™ SARS-CoV-2 Verification Panel, SeraCare, MA, USA; material # 0505-0129)

The LoD was determined as the lowest concentration that was detected ≥ 95% of the times (i.e., concentration at which at least 19 out of 20 replicates tested positive).

Cross-reactivity was analysed in silico and *wet lab: *Human coronavirus 229E, Human coronavirus OC43, Human coronavirus HKU1, *Human coronavirus NL63, SARS-coronavirus, MERS-coronavirus, *Adenovirus, Human Metapneumovirus, Parainfluenza virus 1-4, *Influenza A &B, *Enterovirus, *Respiratory syncytial virus, *Rhinovirus, *Chlamydia pneumoniae, Haemophilus influenzae, Legionella pneumophila, Mycobacterium tuberculosis, Streptococcus pneumoniae, Streptococcus pyogenes, *Bordetella pertussis, *Mycoplasma pneumoniae, Pneumocystis jirovecii, Candida albicans, Pseudomonas aeruginosa, Staphylococcus epidermis, Streptococcus salivarius.

Name and address of manufacturer:

ender diagnostics ag, Freiburgstrasse 251, 3018 Bern, Switzerland

Article number	EM16S	EM16M	EM16L
Number of tests	80	240	400

Storage: Tubes "1", "2", "pos" and "lys" at ≤-20 °C; use only until expiry date indicated on test kit packaging

Materials provided by the user:

- Pipettes (adjustable) and sterile pipette tips
- 1.5 ml reaction tubes
- Molecular grade water, RNase and DNase free
- Ice to keep samples and reagents cold during preparation
- Clean work surface
- Vortex if available
- Thermoshaker
- Desktop centrifuge
- rtPCR thermocycler programmable to the above-mentioned specifications
- plasticware recommended by rtPCR thermocycler manufacturer

References:

[1] Y Zhao, F Chen, Q Li, L Wang, C Fan, Isothermal Amplification of Nucleic Acids. Chemical reviews, (2015), 115 (22), 12491–12545

[2] NCBI Reference Sequence: NC_045512.2, Bases 2720-8554

[3] L Zou, F Ruan, M Huang, et al., SARS-CoV-2 viral load in upper respiratory specimens of infected patients. N Engl J Med (2020) published online Feb 19.

[4] Institute Pasteur, Paris. 2020. Protocol: Real-time RT-PCR assays for the detection of SARS-CoV-2. https://www.who.int/docs/default-source/coronaviruse/real-time-rt-pcr-assays-for-the-detection-of-sars-cov-2-institut-pasteur-paris.pdf?sfvrsn=3662fcb6_2

Symbols:

CE IVD — CE marking; In-vitro Diagnostics

Read instructions for use

LOT — Batch code

REF — Product reference

Σ — Number of tests

Acceptable temperature range

Legal manufacturer

Expiry date

CE IVD