DBdirect™ RT-PCR Probe Mix SuperSens
DB-1267

General description

DBdirect™ RT-PCR Probe Mix SuperSens One-Step RT-qPCR system is designed for use in TaqMan probe-based, super sensitive real-time quantification of RNA transcripts and viral RNA. Thanks to an outstanding antibody-mediated, hot-start Taq DNA polymerase, this mix is capable of single copy target detection even in challenging multiplexes, which tend to create primer-dimers. DBdirect™ RT-PCR Probe Mix SuperSens further contains a thermostable reverse transcriptase (mutated M-MLV), RNase inhibitors, dNTPs, $MgCl_2$, enhancers and stabilizers.

DBdirect™ RT-PCR Probe Mix SuperSens is suitable for ultrasensitive detection in purified RNA, which can be prepared either manually or automatically using standard column based or magnetic bead bases approaches. Its proprietary composition allows also for direct detection of RNA in biological samples such as human serum, saliva, or cell cultivation media (user must validate its use in his/her application; example of direct detection of viral RNA in cell culture is described in 10.1021/acsinfecdis.0c00829).

Kit Components

Kit component	REF code	Volume (µL)			Storage temperature	Cap colour
		100 rxns	1000 rxns	5000 rxns		
Enhancer mix (4x) SuperSens	RF07975	500	5 000	5 x 5 000	-20 °C	
DBdirect™ RT-PCR Enzyme mix (4x) SuperSens	RF01034	500	5 000	5 x 5 000	**-80 °C**	
PCR grade water	RF08842	1 000	10 000	5 x 10 000	-20 °C	

Storage

Keep DBdirect™ RT-PCR Enzyme mix (4x) SuperSens at -80 °C for long-term storage. Enhancer mix (4x) SuperSens and PCR grade water can be stored at -20 °C. Avoid repeated freezing/thawing, do not exceed four cycles. If you intend to use the components more than once, aliquot them after the first thawing.

All components can be stored separately at 4 °C for up to 2 weeks after the first thawing. However, using the components as soon as possible after thawing and preparing fresh Master mix is recommended.

RT-PCR preparation

Prepare RT-PCR reagents

- Thaw all kit components followed by mixing and collecting the liquid by gently centrifugation of each vial before opening.
- You can keep separate kit components for up to 8 hours at laboratory temperature (25°C) or for up to 2 weeks at 4 °C.
- You can keep Master mix (2x) prepared by mixing Enhancer mix (4x) SuperSens and DBdirect™ RT-PCR Enzyme mix (4x) SuperSens for up to 8 hours at laboratory temperature (25 °C) or for up to 24 hours at 4 °C.

Prepare the Reaction Mix and add an RNA/DNA template/biological sample/cell culture

- Count the total number of reactions in your experiment (including replicates, positive control, negative control, etc.) and calculate the total volume of Master mix with 10% excess.
- Prepare Master mix (2x) from Enhancer mix (4x) SuperSens and DBdirect™ RT-PCR Enzyme mix (4x) SuperSens (Table 1).
- Prepare Reaction mix from Master mix (2x) and primer/probe solution (if you add the same volume of the template to each well, you can prepare mix of Master mix (2x), primer/probe solution and water and put this mix into each well).
- Add RNA, cDNA, DNA template or biological sample of cell culture (or controls) to the wells of the qPCR plate (or PCR tubes) that contains the Reaction Mix, mix gently. Add water to 20 µL, if it is not included in previous step, and mix.
- Note: Pre-heating of biological samples or cell cultures before adding to the final reaction can increase the sensitivity of (RT-)PCR (specific conditions for each individual matrix/medium and target organism need to be tested, e.g. detection of some RNA viruses in transport medium is increased after heating to 65 °C for 15 minutes or to 80 °C for 5 minutes, or in saliva to 80 °C for 10 minutes).
- Seal the qPCR plate with optically transparent foil (or close PCR tubes), briefly centrifuge to remove air bubbles and collect the reaction solution at the bottom of the wells.

Table 1: Composition of the reaction

Component	Final concentration or amount	Volume per 20 µL reaction	Volume per 10 µL reaction*
Enhancer mix (4x) SuperSens	1x	5 µL	2.5 µL
DBdirect™ RT-PCR Enzyme mix (4x) SuperSens	1x	5 µL	2.5 µL
Forward primer(s)	250-1000 nM (each)**	Variable	Variable
Reverse primer(s)	250-1000 nM (each)**	Variable	Variable
Probe(s)	100-250 nM (each)***	Variable	Variable
RNA template/ biological sample, cell culture	2 pg – 100 ng/ 10-20 %****	2-9 µL/ 2-4 µL****	2-4 µL/ 1-2 µL****
PCR grade water		Up to 20 µL	Up to 10 µL

* Recommended volume for use with 384-well qPCR plate.
** 400nM concentration is recommended for the first testing.
*** 100nM concentration is recommended for the first testing.
****10% concentration is recommended for the first testing. Concentration higher than 20 % can inhibit (RT-)PCR reaction.

RT-PCR protocol

Step	Cycles	Temperature	Time (min:sec)
Reverse Transcription	1	50 °C	10:00
Initial denaturation	1	95 °C	02:00
Amplification - Denaturation - Annealing - Extension	45	 95 °C 60 °C * 72 °C	 00:05 00:15 00:15 **
Cooling/Hold if need	1	4 °C	00:30 up to 24 h

* Optimize the annealing temperature based on the primers used.
** In the case of amplification of amplicons longer than 200 bases, extend the time of extension to at least 1 minute per 1 kilobase.

Run PCR

Place the qPCR plate/PCR tubes in the real-time PCR instrument, select detection in appropriate channels based on the probe(s) used and start the cycling program.

Disclaimer

For research use only.

It is the user´s responsibility to validate the specific use of the kit.

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Troubleshooting Guide

High Ct values (> 30) or no amplification curves	
a) PCR inhibitors	Samples of isolated RNA/DNA contain inhibitors from the sample or compounds used during nucleic acid extraction which inhibit PCR. • Perform control measurement of ratios A_{260}/A_{230} and A_{260}/A_{280} to evaluate the purity of RNA/DNA. • Increase extension time. • Add extra steps to the nucleic acid extraction to gain RNA/DNA of higher purity or use lower volume of the RNA/DNA isolate. • It is recommended to use an internal positive control to see the extent of PCR inhibition.
b) Biological matrices/ cultivation media	Biological matrices such as human serum, saliva, cell cultivation media or viral transport media can inhibit PCR. • Pre-heating (specific for individual matrix/medium) before adding to the final reaction can help to increase the sensitivity of (RT-)PCR.

High Ct values (> 30) or no amplification curves	
c) Insufficient amount of template	The amount of template may be too low, it is recommended to add 2 pg - 100 ng of RNA template to reaction. • Quantify the concentration of template using OD_{260} or any fluorescence-based method to ensure using the amount of template as recommended. • If analyzing a sample with unknown concentration add internal control to see whether PCR is not inhibited.
d) Template degradation	RNA/DNA template may be degraded. • Prepare a new sample or a use a different aliquot if available.
e) Primer-dimers or Primer-probe-dimers	Primers and/or probes may form primer-dimers and/or primer-probe dimers. • Try to use lower primer/probe concentration. • Consider a new design of primer(s)/probe(s). • Optimize the annealing temperature.
f) Mispriming	Primers may anneal to the incorrect location on template. • Consider a new primer design. • Increase the annealing temperature.
g) Incorrect annealing temperature	Annealing temperature can be too high. • Optimize the annealing temperature for primers.
h) Short extension time	• Use longer extension time for longer amplicons. It is recommended to set the extension time to 1 min/1 kb.
i) Non-inactivated polymerase	• Include the initial denaturation at 95 °C for 2 mins before the start of the cycling program.
Low fluorescence intensity	
a) Primer-dimers or Primer-probe-dimers	Primers and/or probes may form primer-dimers and/or primer-probe dimers. • Try to use lower primer/probe concentration. • Consider a new design of primer(s)/probe(s). Optimize the annealing temperature.
b) Incorrect probe concentration	• Optimize the probe concentration. It is recommended to use each probe at concentration 100-250 nM.
c) Wrong detection channel	• Ensure the correct detection channel(s) is used in relation to the probe(s) used.
Significant differences in results among replicates	
a) Reagents or reaction mixes not properly mixed	• Mix reagents thoroughly after thawing. Ensure the reaction mixes are mixed well.
b) Sample evaporation	• Make sure the PCR plate is properly sealed to avoid evaporation.
c) Bubbles in reaction wells	• Centrifuge the PCR plate before running PCR to remove bubbles.

No template control (NTC) showing positive result	
a) Contamination	• Discard all contaminated solutions. Work in clean PCR box to avoid contamination of kit components and use PCR grade water provided with the kit. Use tips with filters. • Wells may be contaminated by template from adjacent wells during mixing. • PCR products are a common source of contamination. Keep them separate from PCR reagents.