DB-1270 DBdirect™ PCR Probe Mix
DB-1273 DBdirect™ PCR Probe Mix SuperSens

General description

DBdirect™ PCR Probe Mix and DBdirect™ PCR Probe Mix SuperSens are designed for use in TaqMan probe-based, real-time quantification of DNA sequences. This can be done not only from a purified template but also directly from a range of sample types. Both mixes contain hot-start Taq DNA polymerase (aptamer-mediated in DBdirect™ PCR Probe Mix, and antibody-mediated in DBdirect™ PCR Probe Mix SuperSens), dNTPs, $MgCl_2$, enhancers and stabilizers.

Both DBdirect™ PCR Probe Mix and DBdirect™ PCR Probe Mix SuperSens are well-suited for ultrasensitive detection in purified DNA. The DNA can be purified either manually or automatically using standard column-based or magnetic bead-based approaches. Its unique composition also enables the direct detection of DNA in human cells, bacteria (both gram-positive and gram-negative), viruses (both enveloped and non-enveloped), or direct detection of DNA in different biological matrices such as human serum, saliva, or cell cultivation media (user must validate its use in his/her application). It is also possible to detect DNA from single cells, but a separate preincubation step is needed for some cell types to achieve enough sensitivity. For more information, please refer to the application notes on the product website, which will be updated on a regular basis.

Kit Components

DB-1270 DBdirect™ PCR Probe Mix

Kit component	REF code	Volume (µL)			Storage temperature	Cap colour + label
		100 rxns	1000 rxns	5000 rxns		
PCR Probe mix (2x)	RF05225	1 000	10 000	5 x 10 000	≤ -18 °C	2x
PCR grade water	RF08842	1 000	10 000	5 x 10 000	≤ -18 °C	W

DB-1273 DBdirect™ PCR Probe Mix SuperSens

Kit component	REF code	Volume (µL)			Storage temperature	Cap colour + label
		100 rxns	1000 rxns	5000 rxns		
PCR Probe mix SuperSens (2x)	RF06744	1 000	10 000	5 x 10 000	≤ -18 °C	2x
PCR grade water	RF08842	1 000	10 000	5 x 10 000	≤ -18 °C	W

Storage

Keep all components at ≤ -18 °C for long-term storage. 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.

PCR Probe mix (2x) and PCR Probe mix SuperSens (2x) can be stored at 4 °C for up to 4 weeks after the first thawing. However, using the component as soon as possible after thawing is recommended.

PCR preparation

Prepare PCR reagents

- Thaw all kit components followed by mixing and collecting the liquid by gently centrifugation of each vial before opening.
- You can keep PCR Probe mix (2x) and PCR Probe mix SuperSens (2x) for up to 1 week at laboratory temperature (up to 25 °C). However, it is recommended to run the reaction as soon as possible once you add your primers and probes to the mix (PCR Probe mix SuperSens (2x) is more stable in this condition and can be stored for up to 24 hours with the primers and probes added).

Prepare the Reaction Mix and add the DNA template

- We recommend preparing the components with 10 % excess. It is advantageous to make “master mixes” of several components for multiple reactions whenever possible.
- The recommended reaction volume in 96-well plate is 20 µL: the volume of added PCR Probe mix (2x)/PCR Probe mix SuperSens (2x) is then 10 µL, the volume of other components can be variable – calculate the addition of water in a way that PCR mix (2x) together with primers, probe(s), template(s) will create a 20 µL reaction.
- Note: Reference dye (typically ROX) can be added into the “master mix” (ROX dye is not included in the kit).
- The order of addition of the components is up to you, see Table 1 for details (including concentrations of primers and probes). Maximum volume of biological matrix is 4 µL per 20 µL reaction.
- Note: Pre-heating of biological matrices or media before adding to the final reaction can increase the sensitivity of PCR in some cases (specific conditions for each individual matrix/medium and target organism need to be tested by the user, suitable conditions are e.g., incubating media at 65 °C for 15 minutes or 80 °C for 5 minutes, or incubating saliva at 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[1)]
PCR Probe mix (2x)/ PCR Probe mix SuperSens (2x)	1x	10 µL	5 µL
Forward primer(s)	250-1000 nM (each) [2)]	Variable	Variable
Reverse primer(s)	250-1000 nM (each) [2)]	Variable	Variable
Probe(s)	100-250 nM (each) [3)]	Variable	Variable
DNA template/ biological sample, cell culture [4)]	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

[1)] Recommended volume for use with 384-well qPCR plate.
[2)] 400 nM concentration is recommended if you do not want to optimize the concentration.
[3)] 100 nM concentration is recommended if you do not want to optimize the concentration.
[4)] 10 % concentration is recommended for the first test. Concentration higher than 20 % can inhibit PCR reaction. A quantity of 1 000 cells is recommended for the first test to see if the organism to be tested is suitable for direct PCR.

PCR protocol

Step	Cycles	Temperature	Time (min:sec)
Initial denaturation	1	95 °C	02:00
Amplification - Denaturation - Annealing - Extension	45	95 °C 60 °C [1)] 72 °C	00:05 00:15 00:15 [2)]
Cooling/Hold if need	1	4 °C	00:30 up to 24 h

[1)] Adjust the annealing temperature based on the primers used.
[2)] In the case of amplification of amplicons longer than 250 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 purified 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 DNA. • Increase extension time. • Add extra steps to the nucleic acid extraction to gain DNA of higher purity or use lower volume of the 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 PCR.
c) Insufficient amount of template	The amount of template may be too low, it is recommended to add 2 pg - 100 ng of DNA 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	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. • If you use DB-1270, try DB-1273 which can solve the problem. • 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 inhibitor	• Include the initial denaturation at 95 °C for 2 mins before the start of the cycling program.

<table>
<tr><td colspan="2">Low fluorescence intensity</td></tr>
<tr><td>a) Primer-dimers or Primer-probe-dimers</td><td>Primers and/or probes may form primer-dimers and/or primer-probe dimers.
• If you use DB-1270, try DB-1273 which can solve the problem.
• Try to use lower primer/probe concentration.
• Consider a new design of primer(s)/probe(s).
• Optimize the annealing temperature.</td></tr>
<tr><td>b) Incorrect probe concentration</td><td>• Optimize the probe concentration. It is recommended to use each probe at concentration 100-250 nM.</td></tr>
<tr><td>c) Wrong detection channel</td><td>• Ensure the correct detection channel(s) is used in relation to the probe(s) used.</td></tr>
<tr><td colspan="2">Significant differences in results among replicates</td></tr>
<tr><td>a) Reagents or reaction mixes not properly mixed</td><td>• Mix reagents thoroughly after thawing. Ensure the reaction mixes are mixed well.</td></tr>
<tr><td>b) Sample evaporation</td><td>• Make sure the PCR plate is properly sealed to avoid evaporation.</td></tr>
<tr><td>c) Bubbles in reaction wells</td><td>• Centrifuge the PCR plate before running PCR to remove bubbles.</td></tr>
<tr><td colspan="2">No template control (NTC) showing positive result</td></tr>
<tr><td>a) Contamination</td><td>• 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.</td></tr>
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