DB-1271 DBdirect™ PCR SYBR Mix
DB-1274 DBdirect™ PCR SYBR Mix SuperSens

General description

DBdirect™ PCR SYBR Mix and DBdirect™ PCR SYBR Mix SuperSens contain SYBR™ Green I dye for 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, without the need for TaqMan probes. Both mixes further contain hot-start Taq DNA polymerase (aptamer-mediated in DBdirect™ PCR SYBR Mix, and antibody-mediated in DBdirect™ PCR SYBR Mix SuperSens, dNTPs, $MgCl_2$, enhancers and stabilizers.

DBdirect™ PCR SYBR Mix and DBdirect™ PCR SYBR Mix SuperSens are well-suited for sensitive, resp. 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.

The non-specific nature of SYBR™ Green I dye does not allow for differential detection of multiple targets in one reaction: these mixes are suitable for the detection of a single target in one reaction (monoplex detection), or they can be used for the detection of multiple targets, but they cannot be discriminated from each other during PCR reaction. Melting analysis after the amplification can be run to examine the specificity of amplification.

Kit Components

DB-1271 DBdirect™ PCR SYBR Mix

Kit component	REF code	Volume (µL)			Storage temperature	Cap colour + label
		100 rxns	1000 rxns	5000 rxns		
PCR SYBR mix (2x)[1)]	RF09982	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

[1)] Keep the component away from light.

DB-1274 DBdirect™ PCR SYBR Mix SuperSens

Kit component	REF code	Volume (µL)			Storage temperature	Cap colour + label
		100 rxns	1000 rxns	5000 rxns		
PCR SYBR mix SuperSens (2x) [1)]	RF00683	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

[1)] Keep the component away from light.

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 SYBR mix (2x) and PCR SYBR mix SuperSens (2x) can be stored (in dark) 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.
- Work with PCR SYBR mix (2x) at laboratory temperature (up to 25 °C) for as short a time as possible. You can keep PCR SYBR mix SuperSens (2x) for up 1 week in this condition (in dark). However, it is recommended to run the reaction as soon as possible once you add your primers to the mix (PCR SYBR mix SuperSens (2x) is more stable in this condition and can be stored for up to 24 hours with the primers 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 SYBR mix (2x)/ PCR SYBR 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, 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). 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 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 SYBR mix (2x)/ PCR SYBR mix SuperSens (2x)	1x	10 µL	5 µL
Forward primer	250-1000 nM[2)]	Variable	Variable
Reverse primer	250-1000 nM[2)]	Variable	Variable
DNA template/ biological sample, cell culture[3)]	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)] 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 (tested for amplicons up to 2 kilobases. For longer amplicons, even more time may be required).

Run PCR

Place the qPCR plate/PCR tubes in the real-time PCR instrument, select channel for SYBR™ Green I detection (this dye has excitation maximum at 498 nm and emission maximum at 522 nm, however exact filter setting on your instrument may slightly differ) 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	Primers may form primer-dimers. • If you use DB-1271, try DB-1274 which can solve the problem. • Try to use lower primer concentration. • Consider a new design of primer(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 at least 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.

Low fluorescence intensity	
a) Primer-dimers	Primers may form primer-dimers. • If you use DB-1271, try DB-1274 which can solve the problem. • Try to use lower primer concentration. • Consider a new design of primer(s). • Optimize the annealing temperature.
b) SYBR™ Green I dye degradation	SYBR™ Green I dye in PCR mix (2x) can be degraded. • Use a new aliquot of the component and keep it away from light.
c) Wrong detection channel	• Ensure the correct detection channel for SYBR™ Green I detection is 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	
It is recommended to obtain melting curve analysis to see whether amplicons are intended PCR products or primer-dimers. Primer-dimers generally have lower Tm than PCR products.	
a) Primer-dimers	Primer dimers may lead to undesired amplification visualized by SYBR™ Green I dye in NTC. • If you use DB-1271, try DB-1274 which can solve the problem. • Consider redesigning of primers.
b) 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.