DIRECT HOT START PCR EXTRACLEAR KIT

With Hot Disco taq 2x Master Mix 1 BLUE
1.5 mM $MgCl_2$ final concentration

MADE IN **DENMARK**

Cat. No.: 257730
100 Reactions

-	ExtraClear DNA extraction solution	Hot Disco Taq 2x Master Mix 1, ready to load, 1.5 mM $MgCl_2$
ID No.	**CL10.000-0001**	**CL1.250-0030**
Cap colour	**Clear**	**Red**
Content	1 x 10 ml	1 x 1.25 ml

Product description

Direct Hot Start PCR ExtraClear Kit consists of ExtraClear DNA extraction solution and Hot Disco Taq 2x Master Mix 1, ready to load, which is required for the subsequent PCR.

The ExtraClear DNA extraction solution is designed for rapid and efficient extraction of PCR-ready DNA from various sample types; mammalian tissues (such as mouse tail and ear snips), plant leaves, saliva and bacteria. The non-toxic ExtraClear DNA extraction Solution enables the extraction of DNA from tissues in just 8 minutes. The extraction protocol is divided into two simple heating steps, which is directly followed by PCR using Hot Disco Taq 2x Master Mix 1, ready to load. This method is ideal for PCR analysis such as screening and genotyping.

The one-reagent DNA extraction set-up is easily scaled and can be conducted by robotic automation platforms. Depending on the sample size, the DNA extraction can be performed in PCR tubes or 1.5 ml tubes, using either a thermocycler or heating block.

Hot Disco Taq 2x Master Mix 1 ready to load, is a ready-to-use 2x reaction mix. Each PCR reaction requires 12.5 µl of the master mix. Simply add primers, DNA extract and water to a total reaction volume of 25 µl to successfully carry out PCR.

Hot Disco Taq DNA Polymerase is a modified form of Disco Taq DNA polymerase, which is activated by heat treatment. A chemical moiety is attached to the enzyme at the active site, which renders the enzyme inactive at room temperature. Thus, during setup and the first ramp of thermal cycling, the enzyme is not active and misprimed primers are not extended. The result is higher specificity and greater yields when compared to standard DNA polymerases.

There is no need to use separate loading dyes. Simply load a portion of the reaction product onto an agarose gel for electrophoresis and subsequent visualization. The blue dye front runs at 400 – 500 bp on a 0.5 – 1.5% agarose gel.

Direct Hot Start PCR ExtraClear Kit allows for DNA extraction and amplification here of in less than 2 hours, as compared to ≥1 day with conventional protocols.

Composition of ExtraClear DNA Extraction Solution

- Optimized DNA extraction solution

Composition of Hot Disco Taq DNA Polymerase 2x Master Mix 1, ready to load, 1.5 mM $MgCl_2$

- Tris-HCl pH 8.5, $(NH_4)_2S0_4$, 3.0 mM $MgCl_2$, 0.2% Tween® 20
- 0.4 mM of each dNTP
- Hot Disco Taq DNA Polymerase
- Inert blue dye and stabilizer

Recommended Storage and Stability of Kit Components

ExtraClear DNA Extraction Solution: Long term storage at -20 °C. Product expiry at -20 °C is stated on the label. Can be stored short term at +4 °C for up to 3 months.ExtraClear DNA Extraction Solution tolerates up to 20 freeze-thaw cycles. It is recommended to aliquot the ExtraClear into smaller volumes.

Hot Disco Taq 2x Master Mix 1 ready to load : Long term storage at -20 °C. Product expiry at -20 °C is stated on the label. Can be stored at +4 °C for up to 6 months.

Quality Control

Each batch of ExtraClear DNA Extraction Solution is functionally tested.
Hot Disco Taq DNA Polymerase is functionally tested for contaminating activities, with no traces of endonuclease activity, nicking activity or exonuclease activity.

Extraction Protocol

Preparation of DNA extraction should be performed in a separate area from that used for setting up the PCR.

1. Thaw ExtraClear DNA Extraction Solution. For the first time use, aliquot the ExtraClear DNA Extraction solution into smaller volumes. (ExtraClear DNA Extraction Solution has a cloudy appearance).
2. Add your sample to a tube containing 100 µl ExtraClear DNA Extraction Solution. Recommended sample sizes are shown in Table 1.
3. Vortex the tube containing the sample and the DNA extraction solution for 15 sec.
4. Transfer the tube to a heat block or a thermal cycler and incubate for

    1. 65 °C for 6 min
    2. 98 °C for 2 min
    3. 4 °C (or cool down on ice)

The DNA extract is now ready for PCR. See PCR protocol and table 2.

DNA extracts are stable at -20 °C for one week or long term at -80 °C.

Table 1. Sample sizes

Sample	ExtraClear DNA Extraction Solution	
	100 µl	500 µl
Tissue*	0.5 – 10 mg	10 – 50 mg
Plant**	2 – 10 mg	10 – 50 mg
E. coli	1 colony (Φ 0.5 - 2 mm)	1 colony (Φ 0.5 - 5 mm)
Saliva	10 – 20 µl	50 - 100 µl

* Examples of tested tissues include mouse tail snip, mouse organs and chicken breast.
**Examples of tested plant materials include leaves from stinging nettle and ivy.

PCR Protocol

This protocol serves as a guideline to ensure optimal PCR results when using Hot Disco Taq 2x Master Mix 1 ready to load. Optimal reaction conditions such as incubation times, temperatures, and amount of template DNA may vary and must be determined individually.

1. Thaw Hot Disco Taq 2x Master Mix 1, ready to load, and primers.
   It is important to thaw the solutions completely and mix thoroughly before use to avoid localized concentrations of salts.
   Keep all components on ice.
2. Prepare a reaction mix. Table 2 shows the reaction set up for a final volume of 25 µL. If desired, the reaction size may be scaled up or down.

Table 2. Reaction components (reaction mix and template DNA)

Component	Vol./reaction*	Final concentration*
2x Master Mix	12.5 µl	1x
25 mM $MgCl_2$	Optional	1.5 mM (1.5 – 4.5 mM)
Primer A (10 µM)	0.5 µl (0.25 – 2.5 µl)	0.2 µM (0.1 – 1.0 µM)
Primer B (10 µM)	0.5 µl (0.25 – 2.5 µl)	0.2 µM (0.1 – 1.0 µM)
PCR-grade H_2O	X µl	-
DNA Extract**	2 - 5 µl	Variable
TOTAL volume	25 µl	-

* Suggested starting conditions; theoretically used conditions in brackets
** If the PCR yields are poor or one experience no bands, it might help to dilute the DNA extract 1:10. DNA extracts from plant leaves should be diluted 1:10 or 1:100, especially when analysing chloroplast DNA.

3. Mix gently.
4. Add extracted DNA to the individual tubes containing the reaction mix.
5. Program the thermal cycler according to the manufacturer´s instructions. See table 3 for an example.
   For maximum yield and specificity, temperatures and cycling times should be optimized for each new template target or primer pair.
6. Place the tubes in the thermal cycler and start the reaction.
7. At the end of the run, simply load a portion of the reaction product (e.g. 10 µl) onto an agarose gel for analysis.

Table 3. Three-step PCR program

Cycles	Duration of cycle	Temperature
1	15 minutes	95 °C
25 - 35	20 – 30 seconds 20 – 40 seconds 30 seconds	95 °C 50 – 65 °C 72 °C
1	5 minutes	72 °C

Notes:

- For genotyping of fish fins and other applications please visit our website.

- The final $MgCl_2$ concentration of Hot Disco Taq 2x Master Mix 1, ready to load, is 1.5 mM. In some PCR applications, more than 1.5 mM $MgCl_2$ is required for best results. Use 25 mM to adjust the Mg^{2+} concentration according to table 4.

Table 4. Additional volume (µl) of $MgCl_2$ per 25 µl reaction:

Final $MgCl_2$ conc. in reaction (mM)	1.5	2.0	2.5	3.0	3.5	4.0	4.5
Volume of 25 mM $MgCl_2$	0	0.5	1	1.5	2	2.5	3

For Research Use Only. Not for use in diagnostics procedures.

Other product sizes, combinations and customized solutions are available. Please look at www.dutscher.com or ask for our complete product list for PCR Enzymes. For customized solutions please contact us.

Made in Denmark

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