Xpert directXtract PCR Kit

#GE60.0080 (80 rxns) | #GE60.0480 (480 rxns) | #GE60.1200 (1200 rxns)

(for research only)

Product:

Xpert directXtract PCR kit combines a simple but efficient DNA extraction method with direct amplification using Xpert Fast Hotstart DNA polymerase in a convenient and easy-to-use manner. This kit can be used with a variety of samples, including whole blood, mouse tails, FTA-cards, and FFPE tissue, and is thus ideal for genotyping and screening, eliminating the need of time-consuming and costly DNA purification methods. DNA extraction is carried out in a single tube, without the need of multiple washing steps, therefore minimizing the risk of contamination. DNA is ready for PCR in only 15 minutes! Xpert Fast Hotstart DNA polymerase, with its extension rate of 4-8 kb/min, not only allows for fast PCR, but in combination with optimized buffer ensures high yield and sensitivity. Upon completion of PCR, the reaction is ready for direct loading onto an agarose gel without the need of adding loading buffer.

Contents:

Product	GE60.0080	GE60.0480	GE60.1200
Xpert directXtract buffer A	1.6 ml	6x 1.6 ml	15x 1.6 ml
Xpert directXtract buffer B	0.8 ml	6x 0.8 ml	15x 0.8 ml
Xpert Fast Hotstart Mastermix (2x) with dye (#GE45)	1.0 ml	6x 1.0 ml	15x 1.0 ml

GE60.0080 is suitable for 80 DNA extractions and 80 PCR reactions of 25 µl.
GE60.0480 is suitable for 480 DNA extractions and 480 PCR reactions of 25 µl
GE60.0480 is suitable for 480 DNA extractions and 480 PCR reactions of 25 µl

Applications: Direct PCR, Mouse Genotyping, Genetic Screening, Multiplex PCR

Properties:

Amplicon size: up to 5kb.
Extension Rate 4-8 kb/min
Hotstart: Yes
A-overhang: Yes

Samples:

The amount of required sample depends on the type of sample. Recommended quantities for 100 µl extractions are as follows:

- Mouse Tail: 1 to 2 mm (approx 5 mg)
- Mouse Ear Punch: 2 to 4 mm^2 (approx 5 mg)
- Animal Tissue: 3 to 30 mg
- FFPE Tissue: 1 mm^3 (or 2 mm^2 of a 10 µm section)
- Buccal Swab: 1 swab
- Blood (mammalian): 2 to 8µl of fresh blood (with EDTA as anti-coagulant)
- FTA or Guthrie card: 2 mm^2
- Hair follicles: 1 to 10 follicles

DNA extractions may be scaled up (or down), however, the ratio of sample to extraction volume should remain as indicated above. In case of buccal swabs, if desired, use larger volume per swab (up to 300µl) to increase yield.

QC: Functionally tested in PCR. Absence of endonucleases, exonucleases, and ribonucleases was confirmed by appropriate assays.

Storage: -20ºC for at least 1 year. No loss of performance is detected after 20 freeze/thaw cycles.

Basic Protocol

1.Extraction

Prepare for each sample 100 µl reaction mixture consisting of 70 µl PCR-grade ddH_2O, 20 µl Xpert directXtract buffer A, and 10 µl Xpert directXtract buffer B. Cover each sample with 100 µl of reaction mixture and incubate at 75ºC for 5 minutes (lysis and protein denaturation). Heat-inactivate by incubation at 95ºC for 10 minutes. Centrifuge at 14.000-16.000g for 1 minute to pellet debris and transfer the cleared supernatant to a new DNase-free microtube. Any remaining visible residue will not have a negative impact on the downstream PCR. Store at -20ºC for up to several months, or proceed with PCR directly.

2.PCR

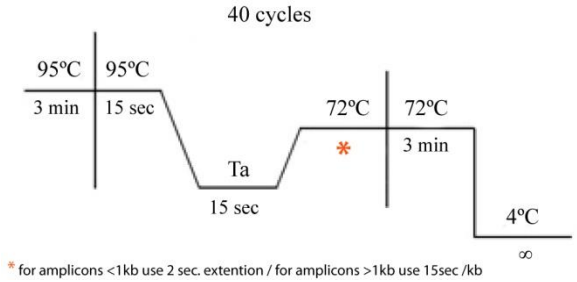
Optimal PCR cycling conditions (incubation times, temperatures, and concentrations) depend on DNA target (GC-content, size, quantity, purity, etc), specific primers, buffer composition, $MgCl_2$-concentration, and the nature and concentration of the DNA polymerase, and need to be determined case by case. Xpert Fast Hotstart Mastermix (2x) with dye includes a fast hot-start DNA polymerase, dNTPs and $MgCl_2$, and has already been optimized for samples treated with Xpert directXtract buffers to maximize success rates. It is not recommended to add additional $MgCl_2$ or other PCR enhancers. We suggest to start with the following basic protocol and subsequently optimize annealing temperature, incubation times and cycling number.

Mix for each PCR reaction according to the following table. In order to minimize risk of contamination, reagent loss and improve pipetting accuracy, we recommend to prepare a mastermix for multiple samples (N), always including a negative control for the detection of possible contaminants, by mixing all components (N+1), except template DNA, dividing the mixture equally into each tube and then add template DNA or PCR grade water in case of the control to the individual PCR tubes. For smaller/larger reaction volumes, scale it down/up proportionally

Component	Volume (25 µl)	Final Conc.
Xpert Fast Hotstart Mastermix (2X) with dye	12.5 µl	1X
Forward primer (5 pmol/µl)	2 µl	0.4 µM
Reverse primer (5 pmol/µl)	2 µl	0.4 µM
Diluted DNA extract*	1 µl	conform sample
PCR–grade water	7.5 µl	

*) Please note that it might be necessary to optimize the amount of template. We recommend to dilute the DNA extract 10-fold with PCR-grade water (e.g. 5 µl of DNA extract + 45 µl ddH_2O) and then use, as indicated in the table above, 1µl as template. This 10-fold dilution ensures the dilution of PCR inhibitors. If the DNA concentration is too low, one can dilute less (e.g. 5-fold or undiluted), however, this might result in a less efficient amplification as the concentration of inhibitors will also be higher and it might be necessary to adapt the cycling conditions (number of cycles and/or extension time). Alternatively, results might be improved if template DNA is diluted even more (e.g. 20-fold).

Set-up initial PCR amplification as follows:



After an initial cycle of 3 min at 95ºC (enzyme activation, denaturation of template DNA), cycle 40 times for 15 seconds at 95ºC, 15 seconds at Ta, and 2 to 75 seconds at 72ºC for extension (2 seconds for targets below 1 kb and 15 seconds per kb for target DNA up to 5kb | 90 seconds total per cycle in case of multiplex PCR, independent of the sizes of the amplicons). Set the annealing temperature (Ta) as the melting temperature (Tm) of the primer with the lowest Tm. Include a final extension step of 3 min at 72ºC to ensure that all amplicons are fully extended and include 3´-A-overhang. Analyze PCR products by DNA Agarose gel electrophoresis. Samples can be loaded directly onto an agarose gel without the need of adding a loading buffer. Using a 1% agarose gel, the inert red tracking dye co-migrates with DNA of approximately 600bp and using a 2% with DNA of approx 350bp.

Optimization

Annealing Temperature (Ta) and Primers

Optimizing the annealing temperature is crucial, especially in case of multiplex PCR, as a too low temperature might result in non-specific amplification whereas a too high temperature results in no amplification. The melting temperature (Tm) is defined as the temperature in which 50% of the primer and its complementary sequence of the target DNA are present as duplex DNA. By increasing the temperature above the melting temperature, this percentage decreases, however, primers will still anneal (up to a certain point) and initiate extension. PCR can therefore be performed at temperatures of several degrees higher than Tm and it is therefore recommended to optimize the Ta by performing a temperature gradient (e.g. starting at the lowest Tm or a few degrees below, and increasing with 2ºC increments). Ideally, primers have melting temperatures of approximately 60ºC and final concentration should be between 0.2 and 0.6µM (each).

Incubation times and number of cycles.

Denaturation and annealing steps may require less time depending on the thermocycler apparatus (mainly ramp rate), reaction volume and PCR tube (varies with the efficiency of heat-transfer). It might be worthwhile to optimize (reduce) times to as low as 10 seconds for both denaturation and annealing steps, which will greatly reduce overall PCR time. **Fast cycling conditions should not be applied in multiplex PCR**. The initial recommended 90 seconds per cycle may be further extended to increase yield. It might be worthwhile to reduce number of cycles from 40 to 25-30, depending on the success of amplification.