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Manual

ExToPCR™ Kit

Fast, enzymatic extraction of DNA from various samples. Kit contains Hot Start PCR Mix.

catalog #	size
1032K-100	100 reactions

For research use only.

Guarantee

A&A Biotechnology provides guarantee on this product.
The company does not guarantee correct performance of this kit in the event of:

- not adhering to the supplied protocol
- use of not recommended equipment or materials
- use of other reagents than recommended or which are not a component of the product
- use of expired or improperly stored product or its components

Table of Contents

Advantages

- Fast, 15 minute procedure.
- DNA extraction is performed in a single-tube, without the need for multiple washing steps or centrifugation.

Specification

form	buffer-based DNA extraction
sample type	• blood • FFPE tissue • swabs • hair follicle • animal tissue • insect • feathers

Description

The ExToPCR™ Kit consists of reagents for DNA extraction and Hot Start PCR mix.

Fast lysis procedure allows for efficient extraction of DNA in an amount required for PCR reactions. Thermostable XTP enzyme and dedicated XTP buffer ensures optimal extraction efficiency and inactivation of cellular nucleases. In addition, the extraction buffer does not contain harmful and irritating substances.

The supplied XTP HS-PCR Mix contains antibody-blocked thermostable DNA polymerase. It is activated during the initial DNA denaturation, which significantly improves the specificity of the reaction. In addition, the XTP HS-PCR Mix contains components to prevent the inhibition of PCR, as well as dyes and loading buffer, which allows the application of samples directly on the agarose.

Contents

component	quantity	storage
XTP buffer	10 ml	-20 °C
XTP enzyme	500 µl	-20 °C
XTP HS-PCR Mix	1.25 ml	-20 °C
ultrapure water	1.5 ml	-20–25 °C

Additional equipment and reagents

Necessary

- 0.2 ml PCR tubes
- Thermoblock or water bath
- Thermocycler

Optional

- TE buffer or Tris-HCl pH 8.0
- 1.5 ml Eppendorf tubes

Sample preparation

sample type	preparation
Blood	1. Add to 0.2 ml tube: ○ **5-10 µl** of fresh or EDTA blood ○ **85 µl** of **XTP buffer** ○ **5 µl** of **XTP enzyme** 2. Follow the extraction protocol.
FFPE tissue	1. Trim all excess wax from FFPE tissue sample 2. Add to 0.2 ml tube: ○ **1 mm³** or **1-2 mm²** fragment of **10 µm** section FFPE tissue sample ○ **85 µl** of **XTP buffer** ○ **5 µl** of **XTP enzyme** 3. Follow the extraction protocol.
Swabs	1. Place cut off swab in 1.5 ml tube and add: ○ **300 µl** of 0.5X **XTP buffer** (diluted in water) ○ **5 µl** of **XTP enzyme** 2. Follow the extraction protocol.

Hair follicle	1. Add to 0.2 ml tube: ○ **1-10** individual follicles ○ **85 µl** of **XTP buffer** ○ **5 µl** of **XTP enzyme** 2. Follow the extraction protocol.
Animal tissue	1. Add to 0.2 ml tube: ○ **2 mm³** tissue fragment ○ **85 µl** of **XTP buffer** ○ **5 µl** of **XTP enzyme** 2. Follow the extraction protocol.
Insects	1. Place an insect in a 1.5 ml tube. 2. Add an **XTP buffer** to immerse the entire insect. 3. Crush the insect with a pipette tip or other sterile tool. 4. Add **5 µl** of **XTP enzyme** 5. Follow the extraction protocol.
Feathers	1. Place **2-5 mm** quill fragment in a 0.2 ml tube and add: ○ **100 µl** of **XTP buffer** ○ **20 µl** of **XTP enzyme** 2. Follow the extraction protocol.

Extraction protocol

1. Close the tube with the sample.
2. Incubate in a waterbath, thermoblock or thermocycler for **10 min** at **50 °C.**
3. Incubate in a waterbath, thermoblock or thermocycler for **5 min** at **95 °C.**
4. Keep the sample at **room temp.** to cool down.

   Note: If the sample fragment does not dissolve completely do not remove it from the tube. Despite the presence of a sample fragment, the DNA present in the extract is safe.
5. Follow up with PCR or store DNA extract at 4 °C for up to 1 month.

PCR protocol

1. Thaw the **XTP HS-PCR Mix** and **sterile water** on ice, gently mix by inverting the tubes and briefly centrifuge. Place the tubes on ice again.

2. Place PCR tubes on ice and add:

component	amount
XTP HS-PCR Mix	12.5 µl
forward primer (10 µM)	0.5-1.25 µl
reverse primer (10 µM)	0.5-1.25 µl
DNA extract	1 µl
ultrapure water	up to 25 µl

3. Gently mix the samples and briefly centrifuge.

4. Place the tubes in the thermocycler and start the PCR with the following protocol.

step	temperature	time	cycles
initial denaturation	95 °C	5 min	1
denaturation	95 °C	15 s	35-40
annealing	40-70 °C *	30 s	
elongation	72 °C	30-60 s / 1000 bp	
final elongation	72 °C	5 min	1

*) The optimal annealing temperature should be calculated using available formulas for annealing temperature (Ta) or determined experimentally.

PCR product analysis

The XTP HS-PCR Mix contains a loading buffer and two dyes: blue and yellow. PCR products can be directly applied onto agarose gel. When running a 2% agarose gel separation, the blue dye migrates as DNA fragments of 1 kb while the yellow dye represents the front of the separation.

Troubleshooting

Thick lysate after DNA extraction

If the lysate is thick or there is a problem with pipetting centrifuge it briefly and use supernatant in PCR. Alternatively dilute lysate 1 : 5 – 10 with TE buffer or Tris-HCl pH 8.0.

PCR inhibition

In case of non-specific or lack of PCR product, dilute the DNA extract 1:5 – 10 with TE buffer or Tris-HCl pH 8.0.

Safety information

DANGER

XTP enzyme

H315 Causes skin irritation.
H319 Causes serious eye irritation.
H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335 May cause respiratory irritation.
P261 Avoid breathing dust.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P342+P311 If experiencing respiratory symptoms call a Poison Center or doctor / physician.

A&A BIOTECHNOLOGY

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A&A Biotechnology, ul. Strzelca 40, 80-299 Gdańsk, Poland
phone +48 58 883 323 761, +48 600 776 268
info@aabiot.com, www.aabiot.com

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