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# PCRBIO Rapid Extract PCR Kit

## Product description:

PCRBIO Rapid Extract PCR Kit combines rapid DNA extraction with fast, highly specific DNA amplification in a convenient, easy to use format. Eliminate the need for laborious and time-consuming DNA extraction methods with this simple, integrated extraction and amplification PCR kit powered by the latest advances in hot-start polymerase technology.

PCRBIO Rapid Extract PCR Kit has been developed for fast, efficient amplification of DNA from a variety of tissues and is particularly suited to solid tissue such as mouse tail or mouse ear. DNA extraction is performed in a single tube, removing the need for multiple washing steps. Extraction of DNA is rapid, providing DNA for PCR in 15 minutes. Extraction takes place in a single tube, minimizing potential contamination.

Extracted DNA is amplified using PCRBIO HS Taq Mix Red. Our antibody-mediated hot start polymerase uses the latest developments in polymerase technology and buffer chemistry to enhance PCR speed, yield and sensitivity. The completed reaction is ready for direct gel loading without the need to add loading buffer.

| Component | 80 reactions | 400 reactions |
|---|---|---|
| 5x PCRBIO Rapid Extract Buffer A | 1 x 1.6 mL | 5 x 1.6 mL |
| 10x PCRBIO Rapid Extract Buffer B | 1 x 800 µL | 5 x 800 µL |
| 2x PCRBIO HS Taq Mix Red | 2 x 1.0 mL | 10 x 1.0 mL |

## Shipping and storage

On arrival the kit should be stored between -30 °C and -15 °C. If stored correctly the kit will retain full activity for 12 months.

## Limitations of product use

The product may be used for in vitro research purposes only.

## Technical support

Help and support is available on our website at https://pcrbio.com/resources/ including answers to frequently asked technical questions. For technical support and troubleshooting you can submit a technical enquiry online, or alternatively email technical@pcrbio.com with the following information:

- Reaction setup
- Screen grabs of qPCR or PCR data.

## Sample amounts

| Sample | Amount per 100 µL extraction | Notes |
|---|---|---|
| Mouse tail clip | 1 to 2 mm (2.5 to 6 mg) | |
| Mouse ear punch | 2 to 4 $mm^2$ (2.5 to 6 mg) | |
| Animal tissue | 3 to 30 mg | |
| Hair follicle | 1-10 individual follicles | |
| Buccal swab | 1 swab | Use 300 µL extraction volume for higher yield |
| Mammalian blood | 2 to 8 µL Fresh/EDTA blood | 2 $mm^2$ FTA, FTA elute or Guthrie cards |
| FFPE tissue | 1 $mm^3$ or 2 $mm^2$ of 10 µm section | |

## Protocol

### 1. Extraction reaction setup

For each biological sample, create the following 100 µL extraction reaction:

| Reagent | 100 µL reaction | Notes |
|---|---|---|
| Mouse tail clip | 1 to 2 mm (2.5 to 6 mg) | See table above for other samples |
| 5x PCRBIO Rapid Extract Buffer A | 20 µL | Lysis buffer |
| 10x PCRBIO Rapid Extract Buffer B | 10 µL | Protease containing buffer |
| PCR grade $dH_2O$ | 70 µL | |

### 2. Extraction reaction incubation

Incubate extraction reaction for lysis, nuclease and protein denaturation, followed by heat-inactivation:

| Cycles | Temperature | Time | Notes |
|---|---|---|---|
| 1 | 75 °C | 5 min | Vortex twice during incubation |
| 1 | 95 °C | 10 min | Deactivates protease |

### 3. Dilute then centrifuge reaction

Add 900 µL PCR grade $dH_2O$ to the deactivated reaction. Centrifuge at high speed in a microcentrifuge for 1 minute to pellet debris. Supernatant can be used directly in PCR or stored at -30 °C to -15 °C.

### 4. PCR Reaction setup

Prepare a master mix based on the following table:

| Reagent | 50 µL reaction | Final concentration |
|---|---|---|
| 2x PCRBIO HS Taq Mix Red | 25.0 µL | 1x |
| Forward primer (10 µM) | 2.0 µL | 400 nM |
| Reverse primer (10 µM) | 2.0 µL | 400 nM |
| Supernatant from step 3 | 1.0 µL to 2.0 µL | variable |
| PCR grade $dH_2O$ | Up to 50 µL final volume | |

Cycle using conditions based on the following table:

| Cycles | Temperature | Time | Notes |
|---|---|---|---|
| 1 | 95 °C | 1 min to 2 min | Initial denaturation and enzyme activation. For colony PCR increase denaturation time to 10 minutes |
| 40 | 95 °C<br>55 °C to 65 °C<br>72 °C | 15 seconds<br>15 seconds<br>1 to 90 seconds | Denaturation<br>Anneal<br>Extension (15 seconds per kb). For multiplex PCR use 90 seconds |

Analyse by agarose gel electrophoresis. The 2x mix contains a red dye for tracking during agarose gel electrophoresis. In a 2% agarose TAE gel the dye migrates at a rate equivalent to 50-100 bp of DNA. In a 1% agarose TAE gel the dye migration rate is equivalent to 200-300 bp of DNA.