

Catalogue	Pack Size*
<a href="#">HS-KIT</a>	2 x 30 mL (400 extractions)

*\*Assuming 75  $\mu$ L extractions.*

*For research and educational use only.*

## Description

The [HotSHOT DNA Extraction Kit](#) provides a rapid, inexpensive, and reliable alkaline lysis method to extract PCR-quality DNA from a wide range of DNA-rich tissues. Suggested target tissues include animal tissues, from mammals to fish to invertebrates. This protocol has also been used successfully to extract fungal, plant, and bacterial DNA, although some samples can be more problematic due to the presence of PCR inhibitors.

The kit uses a dilute alkaline solution that is heated with the sample in a thermocycler (such as Bento Lab) to lyse (break down) cells and release and denature DNA. This solution is then neutralised with a pH buffer solution to lower the pH and stabilise the released DNA. The protocol involves a simple two-step process of cell lysis and neutralisation, taking only 30 once samples are placed in tubes. A one-tube format minimises handling requirements, reducing potential contamination and DNA loss, and allowing sample sizes to be easily scaled upwards as required.

Alkaline lysis extractions are ideal for DNA-rich tissues that are low in PCR-inhibiting substances. Their main advantages are very low cost per sample, ease of use, and scalability from single to hundreds of samples. Because of this they are widely used for applications from routine genotyping to pathogen detection to DNA barcoding. They are not suitable for samples with high concentrations of PCR-inhibiting compounds, or applications requiring the extraction of total DNA, unfragmented DNA, or double-stranded DNA.

## Application Recommendations

- **For use with a wide range of animal tissues, as well as some plant, fungal and plant tissues.**

HotSHOT alkaline lysis has been used to extract DNA from mammal, bird, and fish tissues; from invertebrates, animal parasites, and parasite blood meals, and zooplanktonic eggs. It has also been used to extract DNA from young plant leaves, pollen, fungal liquid and agar cultures, minute fungal fruiting bodies, and fungal pathogens in plant tissue.

- **A simple protocol for rapid extractions, suitable for low to high throughput**

DNA can be efficiently extracted in 30 minutes using only a single 0.2 mL PCR tube per sample. Minimal handling means that reactions can be scaled up from a few extractions to hundreds a day.

- **A low cost solution for projects with small budget but large sample sizes**

Alkaline lysis extractions allows DNA extraction at a fraction of the cost of other extraction methods, saving money for more expensive reagents.

- **Much safer than most DNA extraction reagents**

The HotSHOT Alkaline Lysis Solution is very dilute (25 mM, or 0.1% w/v) and is not considered an irritant or hazardous according to EU and Global Harmonised regulations and classifications. Extractions also do not involve the use of ethanol. It is therefore considerably safer than many other DNA extraction methods involving guanidium salts or chloroform.

- **Not suitable for extracting large amounts of unfragmented high quality DNA**

The HotSHOT alkaline lysis method is not suitable for applications requiring extraction of large quantities of high quality unfragmented DNA. Electrophoresis gels of extracted genomic DNA are likely to be blank, or to only show a faint smear of fragmented DNA and RNA, due to the small quantities of DNA extracted. However, this will not impact PCR results.

## Reagent Composition

**Lysis Solution:** 25 mM NaOH, pH 12.

**Neutralising Buffer:** 100 mM Tris-HCl, 0.5 mM EDTA, pH 8.

## Storage & Stability

Routine storage at room temperature.

Exposure of the Lysis Solution to air will gradually decrease the pH and effectiveness of this reagent over time. Temporary storage for up to 6 months at room temperature should have minimal detrimental effects, if containers and bags are properly sealed.

For longer term storage, test the pH levels before use to check the pH of the Alkaline Lysis Solution.

## Shipping conditions

Shipped at room temperature.

## Safety warnings and precautions

This product and its components are not considered hazardous in their given concentrations. The Lysis Solution contains 0.1% NaOH (25 mM, pH 12), which is below the threshold considered as an irritant according to (GHS Classification in accordance with EU regulation, and UK regulation).

However, as with all scientific reagents this product should be handled and stored with care as standard practice. Wear gloves. Care should be taken to avoid contact with skin or eyes. In case of contact with skin or eyes, wash immediately with water. Keep out of reach of children.

## Quick Start Protocol

Wear gloves, and use sterile equipment and sterile working practices.

1. Make a numbered list of samples, and label a PCR tube per sample with the corresponding sample number using a fine permanent marker.
2. Place 75  $\mu$ L of Alkaline Lysis Solution in a 0.2 mL PCR tube per sample.
3. Subsample 1–2 mm<sup>3</sup> of sample tissue and transfer into each tube, making sure it is immersed in the Alkaline Lysis Solution. Adding excess sample is likely to cause the extraction and/or PCR to fail.
4. Place the tubes in a thermocycler and incubate at 95 °C for 30 minutes using the Heat Block programme.
5. Add 75  $\mu$ L of Neutralising Buffer to each 0.2 mL tube.
6. Use 1–4  $\mu$ L of extract as a DNA template source in a PCR reaction. Extracts from many tissue types (e.g. plant, fungal, invertebrate, feathers, blood) may benefit from dilution by 10x–100x in order to dilute PCR inhibitors prior to use as DNA template.
7. Store sample tubes in a 0.2 mL box or small bag, clearly labelled to avoid confusion. Refrigerate for the short term, or freeze at –20 °C for longer term storage.