



**Description:** Certain compounds in animal tissues inhibit PCR reactions. DirectPCR Lysis Reagents (Patent Pending) contain inhibitors of these PCR inhibitors. Therefore, DNA released in DirectPCR reagents is compatible for one-step PCR genotyping.

### DirectPCR Lysis Reagent (Ear) Cat # 401-E, 402-E

1. For 0.2 cm diameter-ear piece, add 100–200  $\mu$ l DirectPCR Lysis Reagent (Ear) containing freshly prepared 0.2-0.4 mg/ml Proteinase K (Sigma, cat# p6556, not included). **It is important to use 0.5-0.75 ml tube for ears.** Proteinase K is stable in DirectPCR reagents for ~24 hrs. If a small number of ears are processed, and therefore it is difficult to weigh Proteinase K powder, use genomic PCR-quality Proteinase K solution (Viagen, cat #501-PK) at 0.5-1.0 mg/ml (25-50  $\mu$ l Proteinase K solution per 1 ml DirectPCR reagent). See Table 1 for starting conditions.

**NOTE:** Although 100  $\mu$ l DirectPCR is usually sufficient for complete lysis of 0.2 cm ear piece, application of 150-200  $\mu$ l yields more reproducible results because of better mixing efficiency. Compare several different volumes of DirectPCR reagents for best performance.

2. Rotate the tubes in rotating hybridization oven at 55°C for 5-6 hrs or until no tissue clumps are observed. If necessary, rotation can be allowed overnight without loss of efficacy. Complete lysis is important. Since some ear pieces may not be in contact with solutions, re-position once the sample by shaking the bottles containing tubes, preferentially after 2-3 hrs.

**NOTE:** Rotating hybridization oven performs better than rocking plate. DNA fragmentation by prolonged rotation will not influence significantly PCR performance. Use roughly proportional volume of DirectPCR Lysis Reagent for different sized samples.

3. Incubate crude lysates at 85°C for 45 min by floating the whole rack (containing tubes) on a water bath. (Optional) Precipitate hairs by centrifuging for 10 sec before step 4. Crude lysates may be stored at -20°C for 1 year (or at 4°C for 1 week) without losing efficacy.

4. Use 0.5-1.0  $\mu$ l of lysate for 50  $\mu$ l PCR reaction. Eppendorf Hotmaster Taq Polymerase (cat# 954-14-5018), Sigma JumpStart Taq DNA Polymerase (cat# D9307), or Qiagen HotStar Taq DNA polymerase (cat# 203203) is recommended for PCR.

**Rescue of DNA:** DNA in crude lysates can be rescued for further analysis. Add NaCl to a final concentration of 250 mM, and then add 0.7 volume of isopropanol. DNA will form precipitates. Centrifuge at 4°C for 2 min, discard supernatant, wash DNA with 1 ml 70% EtOH, and dissolve DNA in 50  $\mu$ l 10 mM Tris-HCl (8.0). Use 1  $\mu$ l for PCR.

#### Related Products

Products	Description	Cat #	Price (US \$)
DirectPCR-Tail	500 mouse tails (100 ml)	102-T	139
DirectPCR-Ear	500 mouse ears (50 ml)	402-E	139
DirectPCR-Yolk sac	Yolk sac (100 ml)	202-Y	139
DirectPCR-Cell	Cultured cell (100 ml)	302-C	139
Proteinase K Solution	All types of tissues (100 mg)	501-PK	99

## Important Technical Tips

- 1. Complete lysis.** Big tissue clumps should not be observed after digestion. It is recommended to vigorously shake the bottle (containing microfuge tubes) for 2-3 sec anytime, once or twice, after tissues begin to partially dissolve. This will physically disperse partially digested tissues and reposition microfuge tube, in which tails are separated from lysis reagents, thereby facilitating overall lysis efficiency,
- 2. Proteinase K inactivation.** Inactivation of proteinase K by incubating samples at 85C-86C for 45-50 min is critical to protect Taq polymerase from proteinase K.
- 3. Taq polymerase.** We have tested many types of commercially available Taq polymerases. The listed enzymes are recommended for optimal results.
- 4. Tissue size.** The size of tails should be 0.5 cm or slightly smaller. Use a minimal volume (0.5-1  $\mu$ l for 50  $\mu$ l PCR reaction) of crude lysates for PCR amplification. Too much DirectPCR reagents inhibit PCR efficiency.
- 5. Small tubes and evaporation.** To minimize evaporation, use a 0.75 ml tube when the reagent volume is less than 100  $\mu$ l.
- 6. Small samples and dilution.** If the required DirectPCR reagent volume is less than 50  $\mu$ l, dilute the reagent by up to 2-fold with water, while maintaining the same concentration of proteinase K. If the DirectPCR reagent is '2-fold' diluted, apply '2-fold' more crude lysates for PCR reaction.
- 7. PCR machine.** PCR machines are occasionally a source of technical problems.