FISHER MOLECULAR BIOLOGY

FLS-1001T - Mouse Tails Direct PCR Lysis Reagent

Size: 100 mL (500 tails)

Description: Certain compounds in animal tissues inhibit PCR reactions. Straight PCR Lysis Reagents contain inhibitors of these PCR inhibitors. Therefore, DNA released in Straight PCR Lysis reagent is compatible for one-step PCR genotyping.

For 0.5 cm tail, add 200–300 µl Straight PCR Lysis Reagent (Tail) containing freshly prepared 0.2-0.4 mg/ml Proteinase K (not included).

Proteinase K is stable in Straight PCR Lysis Reagent for ~24 hrs.

If a small number of tails are processed, and therefore it is difficult to weigh Proteinase K powder, use genomic PCR-quality Proteinase K solution at 0.5-1.0 mg/ml (25-50 µl Proteinase K solution per 1 ml Straight PCR Lysis Reagent).

See Table 1 for starting conditions.

NOTE: Although 200 µl Straight PCR Lysis Reagent is usually sufficient for complete lysis of 0.5 cm tail, application of 250-300µl yields more reproducible results because of better mixing efficiency. Compare several different volumes of Straight PCR Lysis Reagent for best performance. If tails are not mixed well with solutions, use 0.75 ml tubes.

1. 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 tails may not be in contact with solutions, re-position once the tails by shaking the bottles containing tubes, preferentially after 2-3 hrs.

NOTE: Rotating hybridization oven performs better than rocking plate. Use 0.75 cm tubes for less than 200 µl of Straight PCR Lysis Reagent. DNA fragmentation by prolonged rotation will not influence significantly PCR performance. Use roughly proportional volume of Straight PCR Lysis Reagent for different sized samples.

2. 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.

3. Use 0.5-1.0 µl of lysate for 50 µl PCR reaction.

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 µl 10 mM Tris-HCl (8.0). Use 1 µl for PCR.

Table 1. Suggested starting lysis conditions for mouse tails.

Tails (cm)	Straight PCR (µl)	Dilution (fold)	Lysates (µl) / 50 µl PCR rxn.
0.2~0.3	150-200	2	1.0-2.0
0.4	150-250	1	0.5-1.0
0.5	200-300	1	0.5-1.0

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 85℃-86℃ for 45-50 min is critical to protect Taq polymerase from proteinase K.

3. Tissue size. The size of tails should be 0.5 cm or slightly smaller. Use a minimal volume (0.5-1 μl for 50 μl PCR reaction) of crude lysates for PCR amplification. Too much Straight PCR Lysis Reagent inhibit PCR efficiency.

4. **Small tubes and evaporation.** To minimize evaporation, use a 0.75 ml tube when the reagent volume is less than 100 μl.

5. **Small samples and dilution.** If the required Straight PCR Lysis Reagent volume is less than 50 μl, dilute the reagent by up to 2-fold with water, while maintaining the same concentration of proteinase K. If the Straight PCR Lysis Reagent is '2-fold' diluted, apply '2-fold' more crude lysates for PCR reaction.

6. **PCR machine.** PCR machines are occasionally a source of technical problems.