DirectPCR® Lysis Reagent Tail

Description:

DirectPCR® Lysis Reagent Tail (Patent Pending) was especially developed for the lysis of mouse tail tissue. After a brief heat treatment, the crude lysates are directly used for PCR without time-consuming genomic DNA isolation. Specialized DirectPCR® products are available for the lysis of mouse ear, yolk sac or cultured mammalian cells.

Order Information:

DirectPCR®-Tail	for up to 250 mouse tails (50 ml)	order no. 31-101-T
DirectPCR®-Tail	for up to 500 mouse tails (100 ml)	order no. 31-102-T

Storage:

Store DirectPCR®-Tail protected from light at 4 - 8 °C. The reagent is stable at least 6 months from the date of shipment under these conditions. Crystals may form upon shipment or storage and should be dissolved by short warming to 37 °C prior to use.

Protocol (see TABLE 1):

1. Add 200 - 300 µl DirectPCR®-Tail containing freshly prepared 0.2-0.4 mg/ml Proteinase K (e.g. peqGOLD Proteinase K solution [20 mg/ml], order no. 04-1075) to a 0.5 cm mouse tail piece. Rotate the tube in a rotating hybridization oven at 55 °C for 3 - 16 hours until complete lysis is achieved.

   NOTE: *Although 200 µl DirectPCR®-Tail is usually sufficient for complete lysis of 0.5 cm tail, application of 300 µl usually yields more reproducible results because of better mixing efficiency. It is recommended to compare a couple of different amounts of DirectPCR®-Tail reagents in the initial trials. For small amounts of tissue, DirectPCR® reagent must be diluted with water, and amount of lysate for PCR reactions needs to be increased proportionally. SEE TABLE 1.*

   NOTE: *Proteinase K is only stable for up to 24 hours when added to DirectPCR® reagent. Always use freshly prepared Proteinase K.*

   NOTE: *Rotating hybridization oven generally performs better than rocking plate. Use 0.75 ml tubes for less than 200 µl of DirectPCR®-Tail. Since the lysates are used for PCR without DNA isolation, DNA fragmentation by prolonged rotation will not significantly influence PCR performance. Use roughly proportional volume of DirectPCR®-Tail for different scaled samples. SEE TABLE 1.*

2. Incubate crude lysates at 85 °C for 45 min by floating the whole rack (containing tubes) on a water bath to achieve complete heat inactivation of Proteinase K.
   Centrifuge debris or condensed water for 10 sec before step 3 (optional).

   NOTE: *Crude lysates may be stored for 1 year at –20 °C or for 1 week at 4 °C without losing efficiency.*

3. Directly use 0.5 - 1 µl of lysate per 50 µl genotyping PCR reaction. (see TABLE 1)

[4. Optional: DNA in crude lysates can be efficiently rescued for further purification. Add NaCl to a final concentration of 250 mM, and 0.7 volumes of isopropanol. DNA will come out from an aqueous phase. Centrifuge at 4 °C for 2 min to precipitate DNA. Discard supernatant. Wash DNA with 1 ml 70 % EtOH and dissolve pellet in 50 µl 10 mM Tris-HCl (8.0). Use 1 µl for PCR.]

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TABLE 1: Suggested starting lysis conditions for mouse tails

Tails	DirectPCR® Tail (incl. Proteinase K)	H_2O	Lysates/50 µl PCR rxn.
0.2 - 0.3 cm	150 µl	150 µl	1.0 - 2.0 µl
0.4 cm	200 µl	-	0.5 - 1.0 µl
0.5 - 0.6 cm	200 - 300 µl	-	0.5 - 1.0 µl

Application notes

1. **Complete Lysis**: Mouse tail pieces should not be longer than 0.5 cm and must be completely immersed in lysis reagent. No large tissue fragments should be visible after lysis.

2. **Proteinase K Inactivation:** Inactivation of Proteinase K is achieved by incubation at 85 °C for 45 min. This step is essential to avoid digestion of Taq Polymerase by Proteinase K in PCR reaction, do not skip this step!

3. **Amount of template:** Use as little lysate for PCR reaction as possible, as higher concentrations of DirectPCR® reagent might inhibit PCR reaction. (See TABLE 1).

4. **Tubes and evaporation:** If reaction volume is less than 200 µl during lysis, use 0.75 ml reaction tubes to limit evaporation.

5. **Small sample volume and dilution:** If small amounts of tissue are to be lysed, dilute lysis reagent with water and increase amount of lysate for PCR reaction proportionally. (see TABLE 1)

6. **Trouble-Shooting:** If PCR results do not match expectations, perform positiv control with isolated (pure) genomic DNA, to exclude problems with the thermal cycler, PCR protocol, PCR reagents or reaction method.

Related Products:

Products	Description	Amount	Order No.
DirectPCR®-Yolk sac	Optimized for the lysis of embryonic tissue (yolk sac)	50 ml 100 ml	31-201-Y 31-202-Y
DirectPCR®-Ear	Optimized for the lysis of mouse ear punches	25 ml 50 ml	31-401-E 31-402-E
DirectPCR®-Cell	Optimized for the lysis of mammalian cells (cell culture)	50 ml 100 ml	31-301-C 31-302-C