

DirectPCR® Lysis-Reagent Yolk sac

Description:

DirectPCR® Lysis Reagent Yolk sac is optimized for the lysis of embryonic 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, mouse tail or cultured mammalian cells.

Order Information:

DirectPCR®-Yolk sac	for Yolk sac (50 ml)	Order No. 31-201-Y
DirectPCR®-Yolk sac	for Yolk sac (100 ml)	Order No. 31-202-Y

Storage:

DirectPCR®-Yolk sac should be stored dark 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 (also see TABLE 1):

1. Add 300 µl DirectPCR®-Yolk Sac, containing 0.2 - 0.4 mg/ml Proteinase K (e.g. peqGOLD Proteinase K solution [20 mg/ml], order no. 04-1075) to E12.5 mouse yolk sac. Rotate the tube in rotating hybridization oven at 55 °C for 5 - 6 hours until complete lysis is achieved.

NOTE: It is recommended to compare a couple of different amounts of DirectPCR®-Yolk sac reagent in the initial trials. For smaller samples dilute DirectPCR®-Yolk sac with distilled water and then use proportionally larger volume of lysate for PCR. 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. Since the lysates are used for PCR without DNA isolation, DNA fragmentation by prolonged rotation will not significantly influence PCR performance. Use 0.75 ml tubes for less than 200 µl of DirectPCR®-Yolk sac. Use roughly proportional volume of DirectPCR®-Yolk sac 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.]

TABLE 1: Suggested starting lysis conditions for mouse yolk sac

Samples	Tube size	DirectPCR®-Yolk sac (incl. Proteinase K)	H ₂ O	Lysate/50 µl PCR rxn
E8.5 yolk sac	0.75 ml	80 µl	160 µl	3 - 4.5 µl
E9.5 yolk sac	0.75 ml	80 µl	80 µl	2 - 3.0 µl
E10.5 yolk sac	0.75 ml	80 µl	-	1 - 1.5 µl
E11.5 yolk sac	1.5 ml	200 µl	-	1 - 1.5 µl
E12.5 yolk sac	1.5 ml	300 µl	-	1 - 1.5 µl
E13.5 yolk sac	1.5 ml	400 µl	-	1 - 1.5 µl

Application Notes

- Complete lysis:** Big tissue clumps should not be visible after digestion. Samples must be completely immersed in lysis reagent to achieve complete and efficient lysis.
- 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!
- 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).
- Tubes and evaporation:** To minimize evaporation, use a 0.75 ml tube when the reagent volume is less than 200 µl during lysis.
- Small samples 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)
- 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®-Tail	Optimized for the lysis of mouse tails	50 ml 100 ml	31-101-T 31-102-T
DirectPCR®-Ear	Optimized for the lysis of mouse ear punches	25 ml 50ml	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

This product is distributed for laboratory use only. CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other uses has not been established.