Viagen Biotech, Inc.
3530 Wilshire Blvd. #695
Los Angeles, CA 90010

http://www.viagenbiotech.com/
Tel: 800-649-3764; Fax: 800-649-3785
Email: viagen@viagenbiotech.com

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Store at 4°C

Description: Certain compounds in animal tissues and cells 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 (Cell)
Cat # 301-C, 302-C

1. Suspend cells from a 10 cm plate in **200–300** µl DirectPCR Lysis Reagent (Cell) containing freshly prepared 0.2-0.4 mg/ml Proteinase K (Sigma, cat # p6556, not included). Proteinase K is stable in DirectPCR reagents for ~24 hrs. If a small number of cells 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 µl Proteinase K solution per 1 ml DirectPCR reagent). **NOTE:** For a small number of cells, dilute DirectPCR Reagent with distilled water up to 10 times and then use a proportionally larger volume of lysates for PCR (see Table 1).

2. Rotate the tubes in rotating hybridization oven at 55°C for 5-6 hrs or until no clumps are observed. If necessary, rotation can be allowed overnight without loss of efficacy. Complete lysis is important. For more reproducible results, re-position once the tubes 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 150 µl of DirectPCR Reagent (Cell). 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 µl of lysate for 50 µl 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 µl 10 mM Tris-HCl (8.0). Use 1 µl for PCR.

Table 1. Suggested starting lysis conditions for cultured mammalian cells.

Size of plate (# cells)	Tube size (ml)	DirectPCR-Cell (µl)	Dilution (fold)	Lysates (µl)/50 µl PCR rxn.
96 well*	0.75	50-70	1	1-1.5
96 well**	0.75	60-80	2	2-3
24 well*	0.75	70-100	1	1-1.5
6 well*	0.75	100-140	1	1-1.5
10 cm*	1.5	200-300	1	1-1.5

* Lysis using rotation hybridization oven. ** Lysis using rocking plate. Wash cells in 96-well plate twice with phosphate buffered saline and add 60 µl DirectPCR Reagent (Cell), which has been 2-fold diluted. Incubate at 55°C overnight. Place the 96-well plate in the wet chamber and float the chamber on the 85°C water bath for 1.5 hrs. Pipette up and down several times. Take 1 µl of lysates 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

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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 μl for 50 μl 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 μl.

6. Small samples and dilution. If the required DirectPCR 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 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.