

## DNAMITE<sup>®</sup> for Difficult Cells

### 100 Preparations

#### Protocol

Before you start:

- Prepare 60°C waterbath
- Thaw Proteinase K at room temperature
- If precipitate has formed in Solution LC, incubate the bottle at 60°C for a few mins
- Put cells into a 1.5 ml microtube and add 0.5 ml of Solution LC. Vortex briefly
- Add 20 μl of Solution PK\*. Vortex briefly
- The DNA can now be extracted or the sample can be kept for up to 2 months at room temp.

#### DNA extraction

- Place tube in 60°C waterbath for 1 hr (can be left longer). Vortex briefly
  - If any cell/sample debris is visible after lysis, spin tube at 2,000 rpm for 2 mins
  - Transfer 450 μl of the liquid into a new 1.5 ml tube
  - Add equal volume of CA. Invert tube a few times or vortex briefly
  - Spin tube in a microfuge\*\* at 13,000 rpm for 7 minutes to pellet the DNA
  - Remove the supernatant carefully with a 1 ml pipette tip
  - Re-spin the tube briefly and remove the rest of the liquid with a fine tip
- It is very important to remove all of the liquid**
- Resuspend the pellet in the appropriate volume of TE. The pellet may not be visible!
  - Leave the tube for at least 5 mins\*\*\* at room temp. to re-hydrate the DNA
  - Vortex briefly
  - Incubate tube at 80°C for 5 mins\*\*\*\*
  - Vortex and spin the tube briefly

The DNA is now ready for amplification or it can be stored  
Store the DNA at +4°C short term or at -20°C long term

#### TIPS:

\*Refreeze remainder of Proteinase K

\*\*Place the tube with hinge positioned outwards, so liquid can be removed from the opposite site without disturbing the pellet

\*\*\*Depending on the type and concentration of the DNA, total re-hydration can take from 5 mins to overnight

\*\*\*\*If double stranded DNA is needed (e.g. for DNA estimation using a spectrophotometer, restriction digests etc.) don't do this step, but make sure that all of the liquid is removed

#### Related Products

**MegaMixes** = Clear or blue PCR mixes for instant and accurate PCRs

**TE** = Molecular Grade 10 mM Tris pH 7.5 & 1 mM EDTA pH 8.0

**Just Water** = Molecular grade water in handy sizes

**Supplied as 6 bottles (2 x 25 ml LC, 2 x 25 ml CA & 2 x 15 ml TE) and 2 x 1 ml tubes of PK**  
**Store at room temperature & at -20°C**

**For Research Only**