

# PROTOCOL

DNAmite - For Difficult Cells



Part Number:	2DK-100
Version:	1.0
Storage:	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
Batch Number:	Marked on tube

## 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 can be stored. Store 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