

DNG™ – Plus

DNA Extraction Solution

For Research Use Only

Catalog No.: EX6082

Quantity: 100 ml

Store at RT

Description

DNG™-Plus Solution is designed to isolate double-stranded DNA from human samples. The procedure requires 30-50 minutes and does not require phenol extraction or proteinase digestion. DNA isolation is based on lysis of the cells and subsequent selective DNA precipitation. Finally, the DNA is washed and desalted by ethanol. DNA obtained by this method can be used for all molecular biology procedures (PCR, restriction digestion, cloning, Southern blot, DNA sequencing, etc.). Typical DNA yield is in range of 1.0-5.0 µg from 100µl fresh whole blood.

Other Components needed for 250 prep.

Isopropanol	75ml
Ethanol 75%	250ml
Sterile distilled water	12.5ml

SAMPLE PREPARATION*

-Whole Blood

must be collected in EDTA-coated tube (final concentration (1mg/ml) to prevent clotting and DNA degradation. DNA extracted from heparinized blood cannot be used for PCR.

-Homogenized sputum for detection of M. tuberculosis(LOW PROTEIN CONTENT)*

Place 1.5ml microfuge-tubes contains 100µl of homogenized sputum sample at 95°C water bath for 20min. and then follow the protocol.

-Buccal or Vaginal Swab for detection of bacterial genome

Air-dry the swab for at least 2hr after collection. Place air-dry swab in 1.5ml microfuge-tube, contains 200µl sterile distilled water and incubate at 56°C water bath for 30min. Press the stem end of tube towards the swab two to three time and then remove swab and place sample at 95°C water bath for 10min then follow the protocol.

Protocole

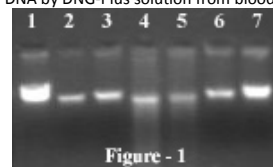
1. Pre-warm DNG™-Plus solution by placing in 37°C for 20min and softly shake.
2. Mix 100µl of sample with 400µl of DNG™-Plus (for whole blood 700µl) and **vortex 15-20sec**. The sample should be completely homogenous suspension at this step. Any aggregation or clot could be degraded by softly pipetting or removed.
3. Add 300µl of isopropanol (for whole blood 500µl), mix by **vortexing**, for low copy DNA samples put in -20°C for 20min (not for whole blood). Then centrifuge the sample at 12,000rpm for 10min.

4. Decant by gently inverting of tube and placing the tube on tissue paper for 2-3sec downward. Care for avoid of cross-contamination between different samples.
5. Add 1ml 75% Ethanol to pellet, mix by **3-5 seconds vortexing** and centrifuge at 12,000rpm for 5min. Repeat this step once more.
6. Pour off the ethanol completely and dry pellet at 65°C for 5min (up to dry).
7. Dissolve DNA pellet in 50µl of sterile distilled water by gentle shaking and placing at 65°C for 5min. Wash the wall of tube for dissolving of any residual pellet by softly pipetting.
8. Pellet unsolved materials by spin 30sec at 12,000rpm, supernatant contains purified DNA.

*Note:

For DNA purification from high protein content, mammalian tissue or sera, use DNP™ Kit (EX6071). Freezing/Thawing of samples should be avoided, since each cycle dramatically diminishes the yield of intact DNA.

Fig.1 (Extracted DNA by DNG-Plus solution from blood on 1% agarose)



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|--------------------------|-------------------------|
| 1- Lambda DNA 48.5Kbp | 5- Six months at 2-8°C |
| 2- five months at -20°C | 6- Two months at 2-8°C |
| 3- Three months at -20°C | 7- Three weeks at 2-8°C |
| 4- Eight months at 2-8°C | |

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