SinaClon BioScience
شرکت سیناکلون

DNP™ Kit

(High yield DNA Purification Kit)

REF **EX6071**

Σ **50 TESTS (30 Tests f whole blood)**

RUO

Components

Contents	Quantity/ Volume	Store Temperature
Protease	250µl	**-20°C**
Protease Buffer	5ml	RT
Lysis Buffer	20ml	RT
Precipitation Buffer	15ml	RT
Wash Buffer	2×50ml	RT
Solvent Buffer	2×1250µl	RT

Description

Easy to use DNP™ Kit is designed to isolate double-stranded DNA from human or animal sources. The procedure requires 35-55 minutes and does not require phenol extraction nor changing tube during procedure. DNA isolation is based on lysis of the cells and subsequent selective DNA precipitation. Finally, the insoluble DNA is washed and desalted by Wash Buffer. DNA obtained by this method can be used for all molecular biology procedures (PCR, restriction digestion, cloning, Southern blot, DNA sequencing, etc.).

Storage and Stability

Store Kit at RT at the first use
Store Protease at -20 °C

Important notes: please read before starting

1. Before use please provide RNase A (Cat. No.: MO5411)
2. Avoid contact any kit reagents with skin & eyes. Wear gloves before use DNP™ Kit. Contact of Lysis Solution with acids or bleach solution, liberates toxic gas. When handling human samples, follow recommended procedures for biohazardous materials.

Protocol

A. Sample Preparation

1. Blood

Whole blood must be collected in EDTA(1mg/ml) to prevent clotting and DNA degradation. DNA extracted from heparinized blood cannot be used for PCR. Typically, 100µl of fresh blood is used for DNA isolation with the yield of 1-5µg. If the blood is to be stored for later it can be left at 2-4°C for no longer than 2 weeks. For long-term storage the samples should be aliquoted in 100µl portions and kept at -20°C.

2. Sera

Add 5µl of Protease to 100µl of serum or plasma in 1.5 ml microcentrifuge tube, vortex and place in 72°C for 10min and then follow the laboratory protocol. Usage of fresh sera samples is recommended. If it is impossible, samples should be frozen in aliquots of 100µl and stored at -20°C.

3. Homogenized sputum for detection of M. tuberculosis

Add 100µl of Protease Buffer to 100µl of homogenized sputum sample placed in 1.5 ml microfuge tube and then add 5µl Protease, mix and then place at 55°C for 30-60 min and then place at 95°C water bath for 20min and follow the protocol.

4. CSF

Centrifuge 1-1.5ml of CSF specimen at 10000g for 5min and discard supernatant. Add 100µl Protease Buffer, mix and then add 5µl of Protease place at 55°C for 30min and follow the protocol.

5. Cell Culture

Collect cells by centrifugation and resuspend in 100µl of Protease Buffer. Add 5 µl of Protease to resuspended cells, mix and then place in 55°C for 30-60 min and then follow the laboratory protocol. For the efficient DNA separation from cell components is not recommended to use more cells than $4\text{-}6\times10^6$. Usage of thawed cells is not recommended.

6. Tissues

Add 100µl of Protease Buffer to 25-50mg of mammalian tissue (either fresh or frozen) placed in a 1.5ml microcentrifuge tube and then add 5µl of protease, place in 55°C for 1 to 3hr (up to degrade) and then follow the laboratory protocol. Using thawed samples is not recommended.

7. Bacterial Cultures (for PCR application)

Bacterial culture should be centrifuged for 10min at 7500g. Collect 2×10^9 or 10-20mg of bacterial culture (either fresh or frozen) in a 1.5ml microcentrifuge tube and suspend it in 100µl of Protease Buffer. Add 5µl of Protease to suspension, mix and then place at 55°C for 30min and follow the laboratory protocol. Using thawed cells is not recommended.

8. Buccal or Vaginal Swab for detection of bacterial genome

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

Vortexing while incubation may increase the DNA yield (every 5 minutes for 5 sec)

B. Extraction Protocol

1. pre-warm kit at room temperature before use. Pre-warm Lysis Solution by placing at 37°C for 10min and gently shake.
2. Mix 100µl of sample with 400µl of Lysis Solution (700µl for whole blood) and vortex 15-20 sec. The sample should be completely homogenous suspension at this step. Any aggregation, clot or insoluble materials could be degraded by softly pipetting or removal.
3. Add 300µl of Precipitation Solution (For whole blood add 500µl), mix by vortexing 5 seconds, then centrifuge 12,000g for 10min.
4. Decant by gently inverting of tube and placing the tube on tissue paper for 2-3 sec down ward. Care should be taken to avoid cross-contamination between different samples.
5. Add 1ml Wash Buffer to pellet, mix by 3-5 seconds vortexing and centrifuge at 12,000g for 5min, then decant (for whole blood and tissue sample repeat this step once more).
6. Remove the Wash Buffer completely and dry pellet at 65°C for 5min. (up to dry).
7. Suspend pellet in 50µl of Solvent Buffer (for serum or plasma sample, suspend pellet in 30µl) by gentle shaking and placing at 65°C for 5min. Wash the wall of tube for mixing of any residual pellet by softly pipetting.
8. Precipitate unsolved materials by centrifuge, 30sec at 12,000g, supernatant contains purified DNA. Measure DNA concentration spectrophotometrically or visually after electrophoresis in fresh 1% agarose gel. (fig.1)

1.Lambda DNA 48.5 Kbp
2.Five months at –20˚C
3.Three months at –20˚C
4.Eight months at 2-8˚C
5.Six months at 2-8˚C
6.Two months at 2-8˚C
7.Three weeks at 2-8˚C

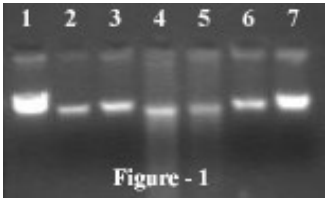

Fig.1 (Extracted DNA by DNP Kit from blood on 1% agarose)

Note

1. For all sample If RNA-free genomic DNA is required, add Ribonuclease A (final concentration 0.2-1mg/ml), to the sample before protease incubation (15min at 37°C).

2. Transcriptionally active tissue and bacterial culture contain high levels of RNA, which can copurify with genomic DNA. RNA may inhibit some downstream enzymatic reactions, but not the PCR itself. If RNA-free genomic DNA is required, add Ribonuclease A (final concentration 0.2mg/ml), to the sample during protease incubation.

3. For whole blood, DNA yield depends on quantity of leukocyte cells and storage duration and condition of sample.

4. Since DNA quantity is too small, Viral DNA from sera samples is invisible in agarose gel and only host nucleic acids from lysed leucocytes may be monitored.

5. Use 1-10µl of DNA solution for each 50µl of PCR mixture. In case of high background PCR product, extracted template DNA may dilute 1/100 and repeat PCR reaction.

Quality Control

All components of the Kit are successfully tested in the DNA purification and amplification reaction for:
Frozen or fresh whole blood for Thalassemia gene, 50mg of stomach biopsy for *H. pylori*, one colony of cultured bacteria for RAPD technique, 100µl of homogenized sputum for MTB and 100µl of positive serum for HBV.

Signs

Signs	Definitions
	Temperature range on product use
	For Research Use Only
	Product shipping conditions
	Name and address of the manufacturer of the product
	Product technical code
	Number of usable tests

Troubleshooting

This guide may help to solve problems that may arise.

Sample	Possible reason	Solution
Low Yield of DNA		
Blood	Sample was frozen and thawed several times. Sample was stored at 2-4°C longer than 2 weeks. Blood clots were present in the sample.	Take new sample.
Cell culture	Too much cells were used for purification, and DNA pellet turned insoluble. Sample was frozen and thawed several times.	Reduce cell quantity twice or more. Take new sample.
Degraded DNA		
Any sample	Inappropriate storage conditions of the sample.	See Sample preparation section.
RNA Contamination Present		
Tissue, bacteria, cell cultures	-	Add of Ribonuclease A to a final concentration 0.2mg/ml to the sample before step 8) and incubate for 10min at 37°C.
Enzymatic Reactions not Running		
Blood	Heparinized blood was used. Deep Red to Black pellet produced in step 5	Use EDTA or citrate treated blood. Pre-warm Lysis Solution at 37°C and repeat extraction
Any sample	Residual reagents (Wash buffer, salts, etc.) present in prepared DNA, because of inaccurate handling during step 5.	Repeat extraction with once more Wash Buffer tretment.

شکایات مشتری

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