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## UptiDNAPure for animal tissues

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### Product Description

**Catalog number:** UPS54631 (25 reactions), UPS54641 (50 reactions) , UPS54651 (100 reactions)

**Name :** UptiDNAPure Genomic DNA Purification kit - from human & animal tissues is designed to recover highly pure genomic DNA, eliminating different contaminating products such as proteins, cellular debris, etc. It is based on a silica matrix, that binds DNA in the presence of chaotropic salts. The DNA bound to the matrix is then eluted in low-salt solutions, ready to be used for downstream applications (Southern blotting, amplification, cloning, labelling, etc.). There is no need to use phenol and precipitation with ethanol/isopropanol

#### REAGENTS INCLUDED IN THE KIT

- Blood Buffer. Store at RT.
- Buffer B. Store at RT. If a precipitate is observed, heat at 50°C
- Proteinase K. Store at 4°C
- Solution I and Blood Buffer. Store at 4°C
- Silica Matrix. Store at 4°C. Never freeze or store under 2°C
- Wash Solution. Store at 4°C
- RNase A. Store at 4°C

#### REAGENTS NOT INCLUDED IN THE KIT

- $\beta$ -mercaptoethanol. Store at 4°C
- Chloroform:isoamylalcohol (24:1). Store at 4°C
- Elution Buffer (TE or double-distilled water).

### Instructions for use

#### I. ISOLATION OF GENOMIC DNA FROM WHOLE BLOOD (500 $\mu$ l)

1. Add to sample 1 ml of Blood Buffer. Mix by gentle inversion. Incubate 10 min at 4 °C.
2. Centrifuge 3 min at 13000 rpm. Discard supernatant.
3. Add 1 ml of Lysis Buffer. Mix by gentle pipetting.  
*Prepare Lysis Buffer before addition to the sample, by adding 15  $\mu$ l of  $\beta$ -mercaptoethanol and 20  $\mu$ l of Proteinase K (10 mg/ml) to 1 ml of Buffer B.*
4. Gently mix the contents of the vial until mixed. Incubate 10-20 min at 65 °C.
5. Centrifuge at 13000 rpm for 5 min. Transfer supernatant to a fresh vial.
6. [OPTIONAL: add 5  $\mu$ l RNase A (10 mg/ml). Incubate 20 min at 37 °C.]
7. Add 1 volume of Solution I + 80  $\mu$ l Silica Matrix (mix well by pipetting before and after addition). Do not vortex.
8. Incubate 10 min at room temperature.
9. Centrifuge 3 min at 10000 rpm. Discard supernatant.
10. Add 700  $\mu$ l Wash Solution (at 4 °C). Resuspend by pipetting.
11. Centrifuge 3 min at 10000 rpm. Discard supernatant.
12. Repeat 10 and 11.
13. Carefully remove all traces of supernatant. It is possible to let the pellet air-dry for 5 min, taking care that the silica matrix does not completely desiccate (this will cause irreversible binding of DNA to the matrix).
14. Add 150  $\mu$ l of elution buffer (TE or sterile double-distilled water), and resuspend thoroughly.
15. Incubate 15-20 min at 65 °C, mixing occasionally.
16. Centrifuge 3 min at 10000 rpm.
17. Transfer DNA-containing supernatant to a fresh vial, without disturbing the silica pellet. If necessary, repeat 16.
18. DNA should be stored at -20 °C. After long storage, it may be necessary to resuspend the DNA, by incubation 1 h at 65 °C, 3 h at 37 °C or O/N at 4 °C.

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## II. Isolation of DNA from whole blood (scalable protocol)

Follow protocol I, using the following amounts:

	25 $\mu$ l blood 1	50 $\mu$ l blood 2	5 ml blo od	10 ml blood
Blood Buffer	50 $\mu$ l	100 $\mu$ l	10 ml	20 ml
Lysis Buffer	50 $\mu$ l	100 $\mu$ l	10 ml	20 ml
RNase A (optional)	1 $\mu$ l	2 $\mu$ l	200 $\mu$ l	400 $\mu$ l
Solution I	1 volume	1 volume	1 vol um e	1 volume
Silica Matrix	20 $\mu$ l	40 $\mu$ l	1 ml	2 ml
Wash Solution	200 $\mu$ l	400 $\mu$ l	10 ml	20 ml

<sup>1</sup> For volumes 3-10  $\mu$ l, the UptiDNAPure for human blood samples provides a fast and economical purification protocol, rendering enough DNA for up to 5 DNA amplification reactions.

<sup>2</sup> For volumes > 500  $\mu$ l, the UptiDNAPure kit for animal tissues provides a fast and economical purification protocol, rendering DNA suitable for a high variety of downstream applications (Southern blotting, amplification, etc.)

## III. Isolation of genomic DNA from cultured cells

1. Harvest the cells and transfer them to a 1.5 ml vial (adherent cells need previous trypsinisation).
2. Centrifuge 5-10 min at 1500 rpm. Discard supernatant.
3. Wash the cells with 200  $\mu$ l cold 1X PBS. Repeat 2.
4. Add 1 ml of Lysis Buffer. Mix by gentle vortexing.

*Prepare Lysis Buffer before addition to the sample, by adding 15  $\mu$ l of **b**-mercaptoethanol and 20  $\mu$ l of Proteinase K (10 mg/ml) to 1 ml of Buffer B.*

5. Gently mix the contents of the vial until mixed. Incubate 10-20 min at 65 °C.
6. Follow with step 5 in protocol I. Note: RNase A step is recommended.

## IV: Isolation of genomic DNA from animal tissues (10-500 mg)

1. OPTIONAL: If necessary, wash the tissue in ice cold 1X PBS to avoid blood contamination. After washing discard PBS.
2. Add 1 ml of Lysis Buffer and homogenise the tissue at 4° C. Optimal homogenisation is a critical step to obtain high DNA yields.

*Prepare Lysis Buffer before addition to the sample, by adding 15  $\mu$ l of **b**-mercaptoethanol and 20  $\mu$ l of Proteinase K (10 mg/ml) to 1 ml of Buffer B.*

3. Incubate 1 hour at 65 °C. Depending on tissue and yields required, incubation time can be extended up to 3 hours.
4. Centrifuge at 13000 rpm for 5 min. Transfer supernatant to a fresh vial.
5. (RECOMMENDED) Add 5  $\mu$ l RNase A (10 mg/ml). Incubate 20 min at 37 °C.
6. Add 0.7 vol of chloroform:isoamylalcohol (24:1), mix well and centrifuge 10 min at 12.000 rpm to separate the phases. Transfer aqueous phase into a fresh vial and follow protocol I in step 7.