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chemagic DNA Tissue 40 Kit *for general purposes*

DNA purification from tissue samples

Kit Components

Magnetic Beads	9.5 ml
Lysis Buffer 1	10 ml
Binding Buffer 2	29.5 ml
Wash Buffer 3	50 ml
Wash Buffer 4	50 ml
Wash Buffer 5	60 ml
Elution Buffer 6	10 ml

The **Elution Buffer** is 10 mM Tris-HCl pH 8.0; one can also use TE buffer pH 8.0.

This kit contains enough materials for 50 isolations from 40 mg tissue (e.g. 1 - 1.5 cm mouse tail section) and is optimized for use with **chemagic** Magnetic Separators.

Completion time: Approximately 30 minutes (without lysis)

Expected yield:

- 1 - 1.5 cm (40 mg) mouse tail: 60 - 90 µg DNA
- 40 mg Liver: 60 - 100 µg DNA

Required Materials

- **chemagic** Magnetic Separator (Magnetic Particle Separator)
- Proteinase K stock solution (10 mg/ml in H_20)

Storage Conditions

This kit may be stored at room temperature (15 – 25 °C) and is stable for at least 1 year following delivery.

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Further Questions?

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Samples and Protocol Adjustments

Before use Proteinase K has to be added to Lysis Buffer 1 to a final concentration of 250 µg/ml (e.g. add 25 µl Proteinase K stock solution (10 mg/ml) to 1 ml Lysis Buffer 1).

The **Elution Buffer** included in this kit is 10 mM Tris-HCl pH 8.0. TE buffer pH 8.0 can also be used without any protocol adjustments. Water pH 8.0 may also be used, in this case we recommend an elution time of 10 - 15 minutes at 55 °C to ensure the highest yield of purified DNA.

UV Measurements

In some cases the manual user may find some traces of the magnetic beads left in the eluate after removal from the tube. Such particles will not interfere with PCR and most downstream applications but may increase the background in UV measurements. In such a case, prior to UV analysis, we recommend an additional application of the magnet to the eluate for 2 minutes in order to separate any traces of particles. For pure DNA the expected A_{260}/A_{280} ratio is between 1.7 - 2.0. The A_{260} value should be between 0.1 and 1.0 for accurate readings.

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Purification Protocol for 40 mg Tissue

1. Cut 40 mg tissue (approx. 1 - 1.5 cm mouse tail section) into small pieces, place in a 1.5 ml tube, add 200 µl **Lysis Buffer 1** (**containing Proteinase K**) and incubate with agitation (600 rpm) over night at 56 °C or until lysis is complete. After lysis spin down material that is not lysed (e.g. bones, hairs) and use the supernatant for the next steps. (The lysis time will be decreased by cutting the tissue in small pieces. Working with the not lysed material will not affect the quality of the DNA, but will make the isolation process more difficult).
2. Add 190 µl resuspended **Magnetic Beads**, premixed with 585 µl **Binding Buffer 2**, to the lysate. Mix with 10 pipetting strokes and incubate **10 minutes** at room temperature.
3. Following incubation, place the tube in a **chemagic** Magnetic Separator to draw the **Magnetic Bead / DNA Complex** to the side of the tube. Leave **2 minutes**, then discard supernatant and **remove the tube from the magnet position**.
4. Add 1000 µl **Wash Buffer 3** to the tube and thoroughly resuspend the beads in the wash buffer by pipetting the bead pellet up and down 15 times.
5. Separate the **Magnetic Bead / DNA Complex** in the magnetic separator for **1 minute**. Aspirate and discard supernatant.
6. **Remove tube from the magnet position** and repeat the washing procedure (steps 4 and 5) using **Wash Buffer 4**.
7. After removing the last traces of **Wash Buffer 4**, **leave tube in the magnetic separator**.
8. With the tube in the Magnetic Separator, and the beads attracted to the side of the tube, gently add 1.2 ml (or as large a volume as possible) **Wash Buffer 5**, being careful not to disrupt the pellet. Leave **90 seconds without resuspending the bead pellet** and then carefully remove and discard the supernatant.
9. (Note: resuspension of the bead pellet in **Wash Buffer 5** may reduce the final DNA yield.)
10. Add 200 µl (or another suitable volume) of **Elution Buffer 6** to the tube and thoroughly resuspend the **Magnetic Bead / DNA Complex** by pipetting the pellet up and down 10 to 15 times.
11. Incubate the suspension for 10 minutes at 55 °C, with agitation (1000 rpm) to facilitate complete DNA elution.
12. Following DNA elution place the tube in the Magnetic Separator for 2 minutes or until all the **Magnetic Beads** have separated from the eluate. Transfer the **eluate** containing the purified DNA to a clean tube. (For UV measurements it is recommended to put the tube containing the eluate again in the magnetic separator and leave for 2 minutes).

Further Questions?

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