Instruction Manual Version 1 - 01.14

XL GenDNA Extraction Module

Cat. No. **C03030020** (mc-magme-003)

diagenode

Technical Assistance & Ordering Information

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www.diagenode.com/en/company/distributors.php
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Contents

Kit modules and components

The GenDNA module from Diagenode has been optimized for the preparation of Genomic DNA from cultured cells.

Table 1 (Note: Upon receipt, store the components at the right temperature)

GenDNA Module			
Component	Description	Quantity	Storage
GenDNA Digestion buffer	Detergent, salt and ion chelator included	3 ml	4°C
GenDNA Proteinase K (200x)	200x stock solution	300 µg / 15 µl	-20°C
GenDNA precipitant	Salt included	3 ml	4°C
GenDNA TE	Ion chelator included	3 ml	4°C
GenDNA RNAse (DNAse free)	-	5 µg / 10 µl	-20°C

Protocol

Short protocol

STEP 1. Cell collection and lysis

1. Pellet suspension culture out of its serum-containing medium. Trypsinize adherent cells and collect cells from the flask. Centrifuge at 300 g for 5 minutes at 4°C.
2. Discard the supernatant. Resuspend cells in 5 to 10 ml ice-cold PBS. Centrifuge at 300 g for 5 minutes. Discard the supernatant. Repeat this resuspension and centrifugation step once more.
3. Add **GenDNA proteinase** K (5 µl) to the **GenDNA Digestion buffer** (1 ml) to make Complete Digestion buffer.
4. Resuspend cells in **complete Digestion buffer** (1.5 million cells/ 500 µl).
5. Cell Lysis: Incubate the samples with shaking at 50°C for 12 to 18 hours in tightly capped tubes.

STEP 2. Nucleic acid extraction and DNA purification

1. Thoroughly extract the samples with an equal volume of phenol/chloroform/isoamyl alcohol.

   Incubate samples 10 minutes at room temperature on a rotating wheel.
2. Centrifuge at 1700 g for 10 minutes.
3. Transfer the aqueous (top) layer to a new tube.
4. Thoroughly extract the samples with an equal volume of chloroform/isoamyl alcohol.

   Incubate samples 10 minutes at room temperature on a rotating wheel.
5. Centrifuge at 1700 g for 10 minutes.
6. Transfer the aqueous (top) layer to a new tube.
7. Add 1/2 volume of **GenDNA precipitant** and 2 volumes of 100% ethanol.

8. Recover DNA by centrifugation at 1700 g for 5 minutes.

9. Rinse the pellet with 70% ethanol. Decant ethanol and air-dry the pellet.

10. Resuspend the pellet of DNA at ~1 mg/ml in **GenDNA TE** until dissolved. Shake gently at room temperature or at 65°C for several hours to facilitate solubilization. Store at 4°C.

11. Residual RNA has to be removed at this step by adding 2 µl of **GenDNA RNase** (DNAse-free) per ml of DNA sample and by incubating 1 hour at 37°C, followed by phenol/chloroform extraction and ethanol precipitation (similar to points above).

12. For DNA analysis, run samples in a 1% agarose gel along with DNA size marker to visualize the DNA preparation efficiency.

Detailed protocol

Starting material- Cell culture
If you perform Methylated DNA IP (MeDIP), you will need 1 µg of DNA per IP. Scale accordingly.

- The GenDNA module provides you with an excess of buffer for the preparation of DNA. Sufficient buffer is given for the preparation of several genomic DNA batches, each obtained from 1 to 3x 10e6 cultured cells (see GenDNA STEP 1, and scale accordingly based on your starting material).
- From about 3 million cells, 20 to 30 µg of DNA can be expected (See Table 2).
- It is also possible to start with less cells, keeping in mind that 1 µg of DNA is needed per IP (Table 2).
- Scale volumes accordingly based on the starting material that is available. Then, the number of IPs that can be done will also depend on the amount of prepared DNA that is available (Table 2).
- After DNA preparation, most of the DNA is then sheared to be used in the IP experiment, but remember that some of the DNA and sheared DNA are needed as control for:

  1) DNA preparation efficiency

  2) Shearing efficiency

  3) The IP experiment efficiency: input sample.

Table 2

DNA preparation	Cell number needed	DNA amount expected	DNA to be used in IP
For 1 Methyl DNA IP	0.3x 10e6	2.0-3.0 µg	1 µg
For 10 Methyl DNA IP	1-1.5x 10e6	8-12 µg	10 µg/10IPs
	3x 10e6	20-30 µg	20 µg/20IPs

STEP 1. Cell collection and lysis

Starting material: culture cells

1. Pellet suspension culture out of its serum-containing medium or trypsinize adherent cells and collect cells from the flask. Centrifuge at 300× g for 5 minutes at 4°C.
2. Discard the supernatant. Resuspend cells in 5 to 10 ml ice-cold PBS. Centrifuge at 300× g for 5 minutes. Discard the supernatant. Repeat this resuspension and centrifugation step once more. This step is to wash the cells.
    - Meanwhile, place the GenDNA Digestion buffer at room temperature (RT) and the GenDNA proteinase K on ice (to be used at Point 3. below).
3. Prepare the complete Digestion Buffer by adding 5 µl Gen DNA Proteinase K to 1 ml GenDNA Digestion Buffer.
4. Resuspend cells in complete Digestion buffer.
    - For 1.0 to 1.5 million cells, use up to 500 µl complete Digestion buffer.
    - It might be necessary to use more buffer to avoid viscosity when performing the extractions.
5. Incubate the samples with shaking at 50°C for 12 to 18 hours in tightly capped tubes.

STEP 2. Nucleic acid extraction and DNA purification

6. Thoroughly extract the samples with an equal volume of phenol/chloroform/isoamyl alcohol (Work under a fume-hood).
    - Add 1 volume of phenol/chloroform/isoamyl alcohol (25:24:1).
    - One volume is 500 µl.
    - Incubate the samples at RT for 10 minutes on a rotating wheel before centrifugation. Use gentle rotation and do not vortex.
7. Centrifuge at 1,700× g for 10 minutes.
    - If the phases do not resolve properly, add another volume of GenDNA Digestion buffer omitting proteinase K, and repeat the centrifugation.
    - If there is a thick layer of white material at the interface between the phases, repeat the extraction.
8. Transfer the aqueous (top) layer to a new tube.
    - Increase volume to avoid viscosity if necessary and pipette slowly.
9. Thoroughly extract the samples with an equal volume of chloroform/isoamyl alcohol.
10. Incubate for 10 minutes at room temperature.
11. Centrifuge at 1,700 g for 10 minutes.
12. Transfer aqueous layer to a new tube.
13. Add 1/2 volume of GenDNA precipitant and 2 volumes of 100% ethanol.
    - One volume corresponds to the original amount of top layer.
10. Recover DNA by centrifugation at 1700× g for 5 minutes.
    - Do not use higher speed to avoid genomic DNA fragmentation.
    - This brief precipitation in the presence of an optimized high salt precipitant (GenDNA precipitant) reduces the amount of RNA in the DNA sample. For long-term storage it is convenient to leave the DNA in the presence of ethanol.
11. Rinse the pellet with 70% ethanol. Decant ethanol and air-dry the pellet.
    - It is important to rinse extensively to remove any residual of salt and phenol.
12. Resuspend the pellet of DNA at ~1 mg/ml in GenDNA TE until dissolved. Shake gently at room temperature or at 65°C for several hours to facilitate solubilization. Store at 4°C.
    - From 1-1.5 million cells, ~8 to 12 µg of DNA can be expected (in a volume of 8 to 12 µl).
    - From 3 million cells, ~20 to 30 µg of DNA can be expected (in a volume of 20 to 30 µl).
    - If possible, it is recommended to get at least 30 µg of DNA (when enough material is available) to be able to work with 30 µg of DNA: see 2/ DNA shearing protocol).
13. Residual RNA has to be removed at this step by adding 2 µl of GenDNA RNAse (DNAse-free) per ml of DNA sample and incubating 1 hour at 37°C, followed by phenol/chloroform extraction and ethanol precipitation.

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Ordering information

Description	Cat. No.
MagMeDIP kit	
MagMeDIP kit x10	C02010020
MagMeDIP kit x48	C02010021
Auto MeDIP kit	
Auto MeDIP kit x100	C02010012
Auto MeDIP kit x16	C02010011
MethylCap kit	
MethylCap kit x48	C02020010
Auto MethylCap kit	
Auto MethylCap kit x48	C02020011

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