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A Geno Technology, Inc. (USA) brand name

GET™ DNA Template

For the Isolation of Genomic DNA Template

(Cat. # 786-353, 786-354)

INTRODUCTION

The GET™ DNA Template kit is suitable for the preparation of DNA Templates from blood, cells fungi and animal tissues and plant tissues. The method involves solubilization in Template Extraction Buffer and rapid purification of genomic DNA with a specific genomic DNA binding spin column. The isolated DNA template is suitable for PCR and other applications. The kit is supplied as a micro kit for 50 preps and the large kit for 100 preps.

ITEM(S) SUPPLIED

Description	Cat. # 786-353 50 preps	Cat. # 786-354 100 preps
Template Extraction Buffer	30ml	2 x 30ml
GET™ Plasmid Mini Columns	1x 50	2 x 50
Wash I	50ml	2 x 50ml
Wash II	20ml	2 x 20ml
TE Buffer	10ml	10ml
LongLife™ RNase	0.5ml	0.5ml

STORAGE CONDITION

The kit is shipped at ambient temperature. Upon arrival, store the kit components as recommended on the label. The kit components are stable for 1 year, if stored properly.

ADDITIONAL ITEM(S) REQUIRED

Ethanol >90% and 70%

PREPARATION BEFORE USE

1. Add 80ml molecular grade ethanol to the Wash II bottle and check the box on the bottle label to indicate ethanol has been added.
2. Equilibrate Elution Buffer to 50-60°C.

PROTOCOL

From Animal Tissue

1a. For optimal yield, rapidly dissect tissue and proceed with DNA extraction immediately or promptly freeze in liquid nitrogen and store at -70°C until required. Add 5-20mg animal ground frozen tissue or fresh diced tissue to a microcentrifuge tube containing 300µl Template Extraction Buffer. Homogenize the sample with a microfuge pestle until a homogenous suspension is acquired. Add an additional 300µl Template Extraction Buffer to bring the total volume to 600µl.

From Cultured Cells

1b. Add up to 2.5 x 10^6 cultured cells per prep to a centrifuge tube. Centrifuge at 1000g for 2 minutes to pellet cells. Remove the supernatant and add 600µl Template Extraction Buffer and vortex until a homogenous mix is achieved. Incubate at room temperature for 10 minutes with agitation.

NOTE*: For attached cells, remove culture medium and add Template Extraction Buffer directly to the cells. For cells in suspension, pellet cells, remove medium, and add Template Extraction Buffer directly to the cell pellet. Do not wash cells. In both cases, draw the cell lysate up and down several times with a narrow bore pipette tip to further disrupt the cells.*

From Non-nucleated blood

1c. Add 50-300µl blood to a centrifuge tube and centrifuge at 5,000xg for 5 minutes to pellet cells, discard supernatant. Add 600µl Template Extraction Buffer and vortex until a homogenous mix is achieved. Incubate at room temperature for 10 minutes with agitation.

From Nucleated Blood:

1d. Add 5-10µl blood to a centrifuge tube and add Template Extraction Buffer to a final volume of 600µl. Vortex until an homogenous mix is achieved. Incubate at room temperature for 10 minutes with agitation.

From Fungal Tissue

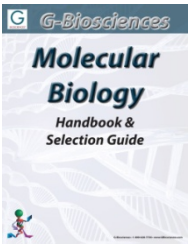
1e. For optimal yield, freeze 50-100mg fungal tissue in liquid nitrogen. Pulverize the tissue whilst frozen to a fine powder. Add 600µl Template Extraction Buffer. Vigorously vortex, incubate at room temperature for 10 minutes.

2. Add 200µl chloroform and vortex for 5-10 seconds to mix. Centrifuge at 10,000xg for 5 minutes to pellet the debris.
3. Carefully pipette the clear supernatant to a clean 1.5ml tube. Add 600µl 70% ethanol to the sample and vortex for 5-10 seconds to mix.
4. Place a GET™ Spin Column into a 1.5ml centrifuge tube and decant the supernatant into the GET™ Spin Column.

5. Centrifuge at 6,000xg for 1 minute to bind the genomic DNA to the matrix. Discard the flow through.
6. Add 0.5ml Wash-I to the column and centrifuge at 6,000xg for 1 minute. Discard the flow through.
7. Add 0.5ml Wash II to the column and centrifuge at 6,000xg for 1 minute. Discard the flow through.
8. Repeat step 7 two more times for a total of 3 Wash II washes. After removing the last wash from the lower tube, briefly centrifuge a final time to remove any residual wash.
9. Transfer spin column to a clean 1.5ml microfuge tube.
10. Vortex the LongLife™ RNase and in a clean tube add 1µl LongLife™ RNase to every 25-50µl 50-60°C TE Buffer for each column.
11. Add the prewarmed RNase:TE Buffer mix directly to the spin column matrix.
12. Incubate at room temperature for 5-10 minutes.
13. Centrifuge at 6,000xg for 1 minute to collect the eluted DNA.
    ***NOTE**: Retain spin column until DNA recovery is checked. If recovery is poor, add 25-50µl hot (50-60°C) TE Buffer to the column and repeat steps 11-12. Combine with previous elution.*

RELATED PRODUCTS

Download our Molecular Biology Handbook.



http://info.gbiosciences.com/complete-molecular-biology-handbook

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