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Rapid™ DNA Template Prep

For the Isolation of DNA Template using pinkRESIN™

(Cat. #786-014,786-015)

INTRODUCTION

The Rapid™ DNATemplate Prep kit is suitable for the preparation of DNA Templates from blood, cells, animal tissues and plant samples. The method involves solubilization of a sample in Template Extraction Buffer. DNA is then selectively bound to *pink*RESIN™. After washing in a spin column, the DNA Template is eluted from the *pink*RESIN™ with a small volume of an elution buffer. The isolated Template is suitable for PCR and other applications.The kit is supplied as a Micro kit, suitable for 50 preps and the Large kit, suitable for 100 preps.

ITEM(S) SUPPLIED (Cat. # 786-014, Cat. # 786-015)

Description	**MICRO**	**LARGE**
Template Extraction Buffer	30ml	2 x 30ml
pinkRESIN™	2 x 1ml	4 x 1ml
Wash I	50ml	2 x 50ml
Wash II	20ml*	2 x 20ml*
TE Buffer	10ml	10ml
LongLife™ RNase	0.5ml	0.5ml
Spin Columns	50 micro	100 micro

** SEE Preparation Before Use*

STORAGE CONDITIONS

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.

ITEMS NEEDED NUT NOT SUPPLIED

- 70% Ethanol
- Absolute Ethanol

PREPARATION BEFORE USE

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

General Notes

Read all steps of the protocol and all application notes before starting. All steps should be carried out at room temperature unless otherwise indicated.

PROTOCOL

Extract and Bind DNA Template to pinkRESIN™

1. The protocol should be carried out in 1.5ml microfuge tubes.
2. Grinding. Pipette 300µl Template Extraction Buffer into a 1.5 ml microfuge tube. Add 1-10 mg animal tissue or 50-100 mg plant sample to the tube and grind it with a clean pestle. For grinding accessories see Application Note. Grind the tissue until completely dispersed. Add 300µl additional Template Extraction Buffer to bring the total volume to 600µl.
3. Blood sample: Use 5-300µl blood Sample. Centrifuge to pellet the blood cells. Remove and discard supernatant. Add 600 µl Template Extraction Buffer. Vigorously vortex the tube.
4. Cells in culture: Add 600µl Extraction Buffer per 1-2 million cells. (See Application Notes for further instructions).
5. Plant and fungal tissues: Most plant and fungal tissues are best prepared by freezing in liquid nitrogen. Pulverize samples while frozen into a fine powder and quickly add an appropriate volume of Template Extraction Buffer.
6. After homogenization, let the sample incubate at room temperature for 10 minutes to complete the tissue lysis.
7. Add 200µl chloroform to the tube and vortex for 5-10 seconds to mix. Invert the tube a few times to complete the extraction. Centrifuge at 10,000xg for 5 minutes to pellet the debris.
8. Carefully pipette the clear supernatant to a clean tube. Add an equal volume (600 µl) of 70% ethanol to the sample. Do not mix at this point.

Prepare pinkRESIN™

9. Vigorously vortex the *pink*RESIN™ tube to re-suspend the resin. Pipette 40µl *pink*RESIN™ to each tube using a large bore pipette tip. Invert to mix and incubate the sample at room temperature for 5 minutes. Keep the *pink*RESIN™ suspended by inverting the tube several times.
10. Centrifuge at 3,000xg for 2 minutes to pellet the *pink*RESIN™ and decant and discard the supernatant. Tap or flick (do not vortex) the tube to re-suspend the *pink*RESIN™ in the remaining small volume of the supernatant.

WASH

11. Add 0.5 ml Wash-I to each tube and tap the tube to re-suspend the *pink*RESIN™. Centrifuge at 3,000xg for 2 minutes to pellet the *pink*RESIN™ and decant off the Wash-I. Tap the tube again to re-suspend the pellet.
12. Add 500 µl Wash II to each tube (use only Wash-II that contains added ethanol). Place a spin column in a clean tube. Re-suspend the *pink*RESIN™ and quickly decant or pipette into the spin column. Ensure that any *pink*RESIN™ remaining in the tube is transferred into the spin column. Briefly centrifuge the spin column and remove the Wash-II from the lower tube.
13. Wash the *pink*RESIN™ pellet two more times using the same volume of Wash-II. After removing the last wash from the lower tube, briefly centrifuge a final time to remove any remaining wash from the bottom and sides of the spin column.

ELUTE

14. Transfer each spin column to a clean tube. Add 1µl *Longlife*™ RNase (vortex the vial before use). Add 25-50 µl hot (50-60º C) TE Buffer. Resuspend the *pink*RESIN™. Use a pipette tip to re-suspend the *pink*RESIN™. Incubate at room temperature for 10 minutes. Centrifuge the spin column briefly to collect the DNA.
    IMPORTANT NOTE: Do not discard the spin columns, unless you have checked DNA recovery. If recovery is poor, elute the DNA from the spin column a second time in the same tube. Add 25-50µl hot (50-60º C) TE Buffer and repeat the elution step as described above.

TEMPLATE SIZE DNA

DNA isolated by this method is template size. Larger size DNA can be obtained by modifying the protocol as follows.

During grinding step, be careful to reduce shear damage to genomic DNA. After homogenization, when you add chloroform, do not vortex. Use wide bore pipette tips to transfer DNA.

In Wash & Elution steps, do not use spin column. Instead, perform Wash and Elution steps in microfuge tubes. Add Wash-I and Wash-II directly to the tube. Perform one Wash-I and three washes with Wash-II. Gently suspend the *pink*RESIN™ for washing. Centrifuge and decant the washes. For elution, add elution buffer into the tube, incubate and centrifuge the tube. Collect eluted DNA with a wide bore pipette.

APPLICATION NOTES

Homogenization Techniques

For efficient grinding of small samples, we offer Molecular Grinding Resin™ (G-Biosciences Cat # 786-138). The Molecular Grinding Resins are high tensile micro particles that do not bind nucleic acids, allowing most samples to be processed by hand using inexpensive macro centrifuge tube pestles or a mortar and pestle.

Cultured cells

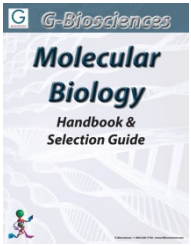
For attached cells remove culture medium and add Template Extraction Buffer directly to cells. For cells grown in suspension, pellet cells, remove medium, and add an extraction buffer to the cell pellet. Do not wash cells. In both cases, draw the cell lysate up and down several times with a narrow bore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

Plant and fungal tissues

Most plant and fungal tissues are best prepared by freezing as described above. Pulverize while frozen to a fine powder and quickly add to the appropriate amount of Template Extraction Buffer.

RELATED PRODUCTS

Download our Molecular Biology Handbook.



http://info2.gbiosciences.com/complete-molecular-biology-handbook

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