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A Geno Technology, Inc. (USA) brand name

Omni DNA Template

PCR Ready DNA Templates - A Single Tube Method

(Cat. #786-013)

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INTRODUCTION

The kit is designed for rapid preparation of DNA Template for large throughput microanalysis, genotyping and other applications. The kit allows high recovery of ready to use DNA Templates in a short period of time. This kit is suitable for 100 preps of 1-10mg sample each.

ITEM(S) SUPPLIED (Cat. # 786-013)

Description	Size
Nuclei Isolation Buffer	2 x 30ml
Templating Buffer	2 x 12.5ml
Longlife™ Proteinase K (5mg/ml)	2 x 0.5ml

SHIPPING & STORAGE CONDITIONS

The kit is shipped at ambient temperature. Upon arrival, store the *Longlife*™ Proteinase K at -20°C and other kit components at 4°C.

PROTOCOL

I. Animal Tissue Samples

1. Transfer a 1-10mg tissue samples into a 1.5ml microfuge tube. Add 0.5ml Nuclei Isolation Buffer into the tube.
2. Place the tube on ice. Using a microfuge pestle (G-Biosciences Cat. # 786-138P), homogenize and evenly disperse the tissue. For homogenization, gently strike the tissues 10-20 times with a microfuge pestle. Lower and raise pestle into the homogenate. Do not twist the pestle while lowering and raising mortar. Twisting may shear the DNA. Do not proceed to next steps until complete tissue homogenization is achieved. Alternatively, remove larger fragments before proceeding to the next step.
3. Centrifuge for one minute at 15,000xg (or at maximum speed) to pellet the nuclei. Remove and discard the supernatant. For tissue with high blood contamination, wash the pellet once with 250µl Nuclei Isolation Buffer. Centrifuge and discard the supernatant. Be careful not to disturb the pellet. Invert the tube on an absorbent tissue to drain off supernatant.

4. Add 25µl of Nuclei Isolation Buffer. Resuspend the pellet by vortexing or using a pestle and add 250-500 µl of Templating Buffer. Mix the *Longlife™* Proteinase K solution gently by tapping the tube and add 2.5-5µl to each sample (use 1µl *Longlife™* Proteinase K/ 100µl Templating buffer). DO NOT ADD MORE THAN RECOMMENDED VOLUME.
5. Incubate the sample at 60^{o}C for 2-4 hour.
6. Incubate the sample at 95^{o}C for 15 minutes to inactivate the Proteinase K.
7. Centrifuge the tube for 10 seconds to bring the condensation to the bottom of the tube. The Template preparation may be stored at -20^{o}C for a month.
8. Use 5µl of the Template preparation in a 50µl PCR reaction mixture.

II. Blood Sample

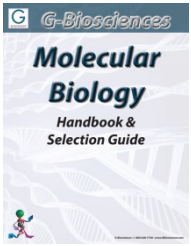
1. Transfer 5-500µl blood samples into a clean microfuge tube. Centrifuge at a high speed. Remove supernatant without disturbing the pellet.
2. Add 0.5 ml of Nuclei Isolation Buffer to suspend the pellet. Incubate at room temperature for 5 minutes.
3. Centrifuge at high speed. Discard the supernatant without disturbing pellet. In case of a blood sample, if there is a blood clot in the pellet remove the clot from the pellet.
4. Repeat steps 2-3 two more times. After last centrifugation, remove and discard the supernatant. Be careful not to disturb the pellet. Invert the tube on an absorbent tissue to drain supernatant.
5. Add 25µl of Nuclei Isolation Buffer. Resuspend the pellet by vortexing. Add 250-500µl of Templating Buffer. Mix the provided Proteinase K solution gently by tapping the tube and add 2.5-5µl to each sample (use 1µl *Longlife™* Proteinase K/100µl Templating buffer). DO NOT ADD MORE THAN RECOMMENDED VOLUME. Incubate the sample at 60^{o}C for 2-4 hours.
6. Incubate the sample at 95^{o}C for 15 minutes to inactivate the *Longlife™* Proteinase K.
7. Centrifuge the tube for 10 seconds to bring the condensation to the bottom of the tube. The Template preparation may be stored at -20^{o}C for a month. Use 5 µl of the Template preparation in a 50µl PCR reaction mixture.

III. For Cell Culture

1. Transfer 10^4-10^6 cells suspension into a clean microfuge tube. Centrifuge at high speed. Remove supernatant without disturbing the pellet.
2. Add 0.5ml of Nuclei Isolation Buffer to suspend the pellet. Vortex for a brief moment
3. Centrifuge at high speed and discard the supernatant.
4. Add 0.25-0.5ml of Templating buffer. Mix the provided Proteinase K solution gently by tapping the tube and add 2.5- 5µl of Proteinase K to each sample (use 1µl *Longlife™* Proteinase K/100µl Templating buffer). **Note:** DO NOT ADD MORE THAN RECOMMENDED VOLUME.
5. Incubate the sample at 60°C for 2-4 hour.
6. Incubate the sample at 95°C for 15 minutes to inactivate the *Longlife™* Proteinase K.
7. Centrifuge the tube for 10 seconds to bring the condensed to the bottom of the tube. The Template preparation may be stored at -20°C for a month.
8. Use 5µl of the Template preparation in a 50µl PCR reaction mixture.

RELATED PRODUCTS

Download our Molecular Biology Handbook.



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