

AutoGenprep 965 Application Guide

ES Cell



- I. **Purpose:** To extract genomic DNA from ES cells.
- II. **Chemical Principal:** Proteinase K / organic extraction method.
- III. **Pretreatment of ES Cells (confluent culture in 24-well plates)**
 - A. Aspirate and dispose of the culture media.
 - B. Wash ES cells with EDTA-PBS buffer.
 - C. Add 0.1ml of [0.25%Trypsin / 0.1%EDTA / PBS] solution in the wells.
 - D. Incubate the plates at 37°C for several minutes.
 - E. Pat the side of plates to get the cells released from the plates.
 - F. Transfer the resuspended cells into 96 deep well plates.
 - G. Load the 96 deep well plates onto the AutoGenprep 965/960.
 - H. Run [Digest] Protocol*. The AutoGenprep 965/960 automatically adds 0.15ml of Reagent M2 (Tissue Digestion Solution 2) and 0.05ml of Reagent M1 (Tissue Digestion Solution 1), containing the pre-dissolved Pro K at the concentration of 1.0mg/ml**) into the plates and mixes them.
 - I. Remove the plates from the 965/960 and incubate overnight at 60-65°C.
* Step 8 and 9 can be done manually.

IV. Protocol Parameters:

- A. **Samples Volume:** Resuspended confluent-cultured ES cells in 24 well plate (0.1ml)
- B. **Maximum Number of Samples:** 384 samples (4 plates) per run.
- C. **Processing Time:** 2.5 hours for 192 samples (2 plates)
4.0 hours for 384 samples (4 plates)
This includes a 20 minutes drying period
- D. **Yield:** Approx. 10 µg of genomic DNA / 1 x 10⁶ cultured cells
The actual yield may vary depending on condition and concentration of starting samples.
- E. **Quality:** OD260/280 values are 1.70 - 1.85
OD230/260 values are < 0.4
The DNA can be used directly in downstream applications such as fluorescent DNA sequencing, PCR, restriction enzyme digestions and more.

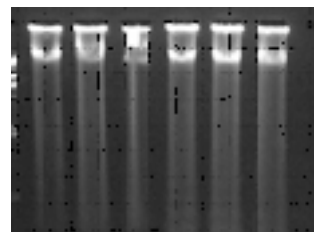
V. Running the Protocol:

- A. Load Reagents and Sample Plates
- B. Select [Extraction] Protocol
- C. Enter Number of Samples
- D. Start the Run

VI. Example of the extracted genomic DNA on the AutoGenprep 965:

Gel electrophoresis of the extracted genomic DNA

Gel: 0.7% agarose, TBE
 Condition: 50V x 60 min.
 Sample: 5 μ l
 M: λ /Hind III marker



VI. Extraction and Purification Process

Process Site	Purpose	System Process
1. Automated or Manual	Digest Tissues	Dissolve ProK with Reagent ES-M1, and add this combined solution with Reagent ES-M2 to samples. Incubate overnight at 60-65°C
2. Automated	Remove RNA	Add Reagents ES-R8 and mix.
3. Automated	Remove Protein & Cellular Debris	Add Reagent ES-R3, mix, centrifuge to pellet debris and transfer supernatant to new (DNA) plate.
4. Automated	Precipitate DNA	Add Reagent ES-R4, mix, precipitate DNA, centrifuge and discard supernatant.
5. Automated	Wash DNA	Add Reagent ES-R5/6/7, mix, centrifuge, and discard supernatant, repeat.
6. Automated	Dry DNA	Dry DNA by heating.
7. Automated	Resuspend DNA	Add Reagent ES-R9 and mix.

AutoGen

84 October Hill Road, Holliston, MA 01746
 Tel: 800-292-5678 or 508-429-5965 | Fax: 508-429-9765
 E-mail: dna@autogen.com