

AutoGenprep 965 Application Guide

Blood Punch



- I. **Purpose:** To extract genomic DNA from blood punch (spot or card) samples
- II. **Chemical Principal:** Modified proteinase K digestion and organic solution chemistry
- III. **Pretreatment of Blood Punch Samples:**
 - A. Place 1 or 2 appropriate size (1/4-1/8 inches or 3-6mm) of blood punches onto a 96 deep-well plate.
 - B. Add 150 μ l of TD-M1 (Tissue Digestion Solution A) containing proteinase K at 0.8mg/ml and 150 μ l of TD-M2 (Tissue Digestion Solution B)
 - C. Seal the plate and incubate at 60°C with consistent shaking for overnight.
- IV. **Protocol Parameters:**
 - A. **Samples Volume:** 0.3ml of digests.
 - B. **Maximum Number of Samples:** 384 samples (4 plates) per run.
 - C. **Extraction Parameters:** A few parameters are modified from standard tissue DNA protocol as follows.
 - Step 10: Centrifuge time is changed to 20min (from 5 min)
 - Step 11: Tip height is changed to 1.0mm (from 3.0mm)
 - Step 21: Drying time is changed to 30 min (from 60 min)
 - D. **Processing Time:** 2.5 hours for 192 samples (2 plates)
4.0 hours for 384 samples (4 plates)
This includes a 30 minutes drying period
- V. **Running the Protocol:**
 - A. Load Reagents and Sample Plates
 - B. Select [Extraction] Protocol
 - C. Enter Number of Samples
 - D. Start the Run

VI. Quality of the extracted total DNA:

A. Spectrophotometric Analysis

Sample#	# of punch	OD260	OD280	260/280	DNA conc. (µg/ml)	Total DNA (µg)
A	2	0.102	0.060	1.69	20.40	1.02
B	2	0.082	0.048	1.71	16.25	0.81
C	2	0.089	0.051	1.75	17.93	0.90
D	2	0.060	0.034	1.76	11.64	0.58
E	2	0.100	0.058	1.72	19.88	0.99
F	2	0.082	0.047	1.73	16.23	0.81

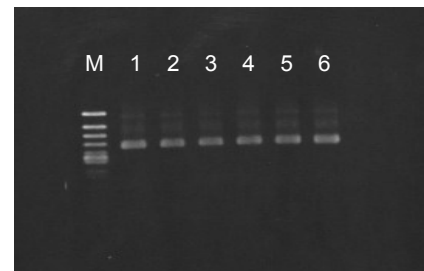
B. PCR

Target: human GAPDH (451 base); Template DNA: 2.5µl of 50µl resuspended DNA solution; Reaction: 94°C, 8min → [94°C,30sec → 59°C,1min → 72°C,1min] x 40 cycles → 72°C,7min

Gel: 1.5% Agarose gel, 1 x TAE

Condition: 100V x 25min.

From left: 1. φ x174/HincII; 2-6. sample A-F



VII. Extraction and Purification Process

Process Site	Purpose	System Process
1. Manual	Digest samples	Add Reagent TR-M1 and TR-M2, and incubate overnight.
2. Automated	Digest RNA	Add Reagents TR-R8 and mix.
3. Automated	Remove Protein & Cellular Debris	Add Reagent TR-R3, mix, centrifuge to pellet debris and transfer supernatant to new (DNA) plate.
4. Automated	Precipitate DNA	Add Reagent TR-R4, mix, precipitate DNA, centrifuge and discard supernatant.
5. Automated	Wash DNA	Add Reagent TR-R5/6/7, mix, centrifuge, and discard supernatant, repeat.
6. Automated	Resuspend DNA	Add Reagent TR-R9 and mix.

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