

Bauer Core Standard Protocol

Title: AutoGenprep 965 Tissue Protocol		
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1. Purpose

This protocol describes how to extract genomic DNA from tissue using the Mouse Tail DNA protocol on the AutoGenprep 965. It is designed as a reference and is not a substitute for training. Users must complete a training session before using any of the Bauer Core's instrumentation.

2. Materials

- 2.1. Up to 50mg of tissue per sample (up to 384 samples in the deep well plates below)
- 2.2. 2ml 96 Deep Well Plate(s) (Corning # 3960)
- 2.3. Tissue DNA Extraction Kit (Autogen # AGKT965TD)
- 2.4. Plate sealers (eg. Hypertask # MPCAL096)

3. Instrumentation - AutoGenPrep 965

4. Reagent preparation

- 4.1 Prepare Protinase K solution (ProK/M1/M2)
 - 4.1.1. Dissolve the protinase K in solution M1.
 - 4.1.1.1. The concentration will depend on how hard the tissue is to digest.
Use 1mg/ml for tissues that are difficult to digest.
Use 0.4 mg/ml for easier tissues.
 - 4.1.2. Mix the protinase K/M1 solution 1:1 with M2 solution.

5. Procedure

- 5.1 Digest the tissue
 - 5.1.1. This step should be optimized for each tissue type to achieve full digestion.
 - 5.1.2. Place up to 50mg of tissue into each well of a 96 deep well plate
 - 5.1.3. Add from 300ul to 500ul of the ProK/M1/M2 solution to each well
 - 5.1.3.1. The plates will be centrifuged and must be balanced.
If the plates are not full, split the samples into the upper rows of two plates.
 - 5.1.3. Digest at 55-60°C from 1h to 16h (until the solution is clear)
- 5.2. Load the Instrument
 - 5.2.1. Make sure the waste containers are empty.
 - 5.2.2. Remove seals from plates containing samples.
Place the sample plates onto the sample rack on the right hand side of the deck
Well A1 should be in the back left corner.
 - 5.2.3. Place an empty deep well plate to the left of each sample plate to receive the purified DNA.
 - 5.2.4. Place tip boxes to the right of the sample plates.
Load one box of tips for reagent dispensing (rear tip box location)
Load one box of tips for each sample plate.
 - 5.2.5. Load the Reagent Reservoirs according to the table below:

Reagent	Reservoir	Vol/Sample (ml)	Sample #			
			96	192	288	384
R3 Deproteinization	3	0.18	42	60	77	94
R4 DNA Precipitation	4	0.20	45	64	83	102
R5/6/7 DNA Wash	5,6,7	0.45	69	112	155	198
R8 Digestion	8	0.05	30	35	40	45
R9 DNA Resuspension	9	0.05	30	35	40	45

- 5.2.5.1. See table for reagent volumes for 96, 192, 288 or 384 samples.
- 5.2.5.2. For other numbers of samples, multiply Vol/Sample by the number of samples and add 25 ml of “dead” volume.
- 5.2.5.3. Reagent 5/6/7 can be used for up to 3 DNA washes
Fill one reservoir for each wash desired (default = 2).

5.3. Run the Software

- 5.3.1. Turn on the Autogen instrument.
- 5.3.2. Open the Autogen software, Select “On Line”, and hit OK.
- 5.3.3. Choose to run a Standard Protocol.
 - 5.3.3.1. Check all the items in the checklist to make sure they are in place.
 - 5.3.3.2. Select the protocol to run – Mouse Tail.
 - 5.3.3.3. Select Automated Run.
- 5.3.4. Choose either the Standard protocol or create a User protocol.
 - 5.3.4.1. Standard protocols can’t be edited except for sample number.
 - 5.3.4.2. Custom protocols can be edited - use to change the # of washes or resuspension vol.
- 5.3.5. Click Start to run the System check.
- 5.3.6. Click OK to begin.

5.4. Clean Up

- 5.4.1. Dispose of all tips in biohazard waste.
- 5.4.2. Dispose of source plates as chemical waste
 - 5.4.2.1. Label the waste as toxic.
 - 5.4.2.2. Record the following waste composition:
 - Chloroform 11%
 - Phenol 5%
- 5.4.3. Empty leftover reagents in the reagent trays into waste bin 2.
 - 5.4.3.1. Rinse the reagent trays with water and add rinse water into waste bin 2.
- 5.4.4. Empty waste bin 2 and dispose of the contents as chemical waste.
 - 5.4.4.1. Label the waste as flammable and toxic.
 - 5.4.4.2. Record the following waste composition:
 - Butyl Alcohol 6%
 - Isopropanol 28%
 - Ethanol 8%
 - Chloroform <1%
 - Phenol <1%
- 5.4.5. Shut down the software and log off the computer
- 5.4.6. Turn off the power to the Autogen instrument.