

AutoGenFlex STAR Application Guide

Buccal Swabs (Tissue Protocol)



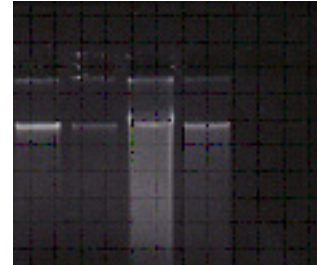
- I. **Purpose:** To extract genomic DNA from buccal swabs.
- II. **Chemical Principal:** Proteinase K/organic extraction method.
- III. **Preparation of Reagents:**
 - A. Add proteinase K to reagent AGF-M1 (Tissue Digestion Solution A) to a final concentration of 0.4mg/ml. This should be prepared fresh before each run.
 - B. If desired, RNase A may be added to reagent AGF-M2 (Tissue Digestion Solution B) in any desired concentration immediately before use. Do not store AGF-M2 with RNase A. The RNase A will degrade during storage.
- IV. **Preparation of Buccal Swabs:**
 - A. Place Buccal Swab in a 15ml Falcon tube (or equivalent). Cut the brush handle short enough so that it does not interfere with the closing of the tube cap.
 - B. Add 250 μ l of AGF-M1 containing Proteinase K and 250 μ l of AGF-M2 to the sample, and incubate at 50-55C (optimal temperature for proK activity) in a shaking incubator at 500rpm for 2-3 hours.
- V. **Protocol Parameters:**
 - A. **Sample Volume:** 0.5 ml of digest solution.
 - B. **Maximum Number of Samples:** 40 samples per run
 - C. **Processing Time:** 3.4 hours for 40 samples (includes 1 hour drying time).
 - D. **Yield:** 2-4 μ g of purified DNA per buccal swab.
 - E. **Quality:** Typical OD260/280 values are 1.8-2.0. The DNA can be used directly in downstream processes such as fluorescence DNA sequencing, PCR, Southern blotting and restriction endonuclease digestions.

VI. Running the Protocol:

- A. Transfer the supernatant from Proteinase K digest to the AutoGen sample tubes. Be sure to remove the buccal swab. Presence of the buccal swab in the AutoGen tube unit will interfere with the extraction process.
- B. Select "Tissue DNA" Protocol on the AutoGenFlex STAR.
- C. Start the run.

VII. Example of genomic DNA obtained from Buccal Swabs:

Samples were reconstituted with 20ul of TE buffer and 10ul was run on a gel. The samples were measured for OD and yielded 2-4ug of DNA with a 260/280 purity of 2.0.



VIII. Extraction and Purification Process

| Process Site | Purpose | System Process |
|--------------|---------------------|---|
| 1. Manual | Digest Proteins | Mix Proteinase K with Reagents M1 and M2, and then add to sample. |
| 2. Automated | Precipitate Protein | Add Reagent R7, mix and centrifuge. Transfer supernatant to DNA tube. |
| 3. Automated | Precipitate DNA | Add Reagent R4, agitate, centrifuge, discard supernatant. |
| 4. Automated | Wash DNA | Add Reagent R5, mix, centrifuge, discard supernatant. Repeat several times. |
| 5. Automated | Evaporate Alcohol | Transfer DNA tube to incubation rack. |
| 6. Automated | Resuspend DNA | Add Reagent R3, mix and centrifuge. |

* All reagents and consumables are sold as a complete kit for Tissue DNA extraction on the AutoGenFlex Catalog #AGKT-FXTD.

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