# BloodPrep™ Chemistry

**Quick Reference Card**

## DNA Isolation from Fresh and Frozen Blood, Tissue Culture Cells, and Buccal Swabs on the ABI PRISM™ 6700 Nucleic Acid Workstation

**⚠ WARNING** Biological samples have the potential to transmit infectious disease. For safety and biohazard guidelines, please refer to the "Safety" section in the *DNA Isolation from Fresh and Frozen Blood, Tissue Culture Cells, and Buccal Swabs Protocol*, P/N 4343586. Follow specific safety practices when using this instrument. For all chemicals in **bold** type below, please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

### Isolation of DNA from Fresh or Frozen Whole Blood

**Prepare samples**

1. Pipette 15 µL of **Proteinase K Solution** (20 mg/mL) in 2-mL microcentrifuge tubes or deep well plates.
2. Add 85 µL of **PK Digestion buffer**.
3. Add 150 µL of blood to the digestion buffer and Proteinase K mixture.
4. Mix thoroughly by pipetting up and down 3 times.

**Lyse whole blood**

1. Incubate mixture at 58 °C for 10 min. Do not allow temperature to exceed 60 °C.
2. Add 500 µL of **BloodPrep DNA Purification Solution** to generate a total of 750 µL.

   Note: If necessary, gently heat **BloodPrep DNA Purification Solution** to 37 °C for 5–10 min to dissolve precipitated salts.
3. Mix the blood and **BloodPrep DNA Purification Solution** thoroughly by pipetting up and down 5 times.
4. *Optional*. Store the lysed samples at 4 °C. If crystallization of salts is observed after storage, gently heat these samples for 5–10 min to 37 °C and then gently vortex.

**Perform purification run**

1. Set up the appropriate purification protocol for the 6700 Workstation.
2. If blood lysate samples are in tubes, pipette into a deep-well plate.
3. Place plate at the secondary input position.
4. Start the run.

### Isolation of DNA from Tissue Culture Cells

**Prepare samples**

- Remove the cells from the tissue culture media.
  - For adherent cells, remove the tissue culture media from all wells by aspiration.
  - For suspension cells, pellet the media by centrifugation and remove the supernatant by aspiration.
- For plasma, serum, and cerebrospinal fluid samples:
  1. Pellet the cells by centrifugation following your standard laboratory procedures.
  2. Remove the supernatant by aspiration.

**Lyse tissue culture cells**

1. Add 200–650 µL of **BloodPrep DNA Purification Solution** into each well of the appropriate 96-well plate.

   Do not exceed 650 µL in volume.

   Note: If necessary, gently heat **BloodPrep DNA Purification Solution** to 37 °C for 5–10 min to dissolve precipitated salts.
2. Mix the cells and **BloodPrep DNA Purification Solution** thoroughly by vortexing for 20 sec to 1.0 min, or by pumping the mixture with a pipette at least 5 times.
3. *Optional*. Store the lysed samples at 4 °C. If crystallization of salts is observed after storage, gently heat these samples for 5–10 min to 37 °C and then gently vortex.

**Perform purification run**

1. Set up the appropriate purification protocol for the 6700 Workstation.
2. Pipette the tissue lysate samples into a deep-well plate.
3. Place plate at the secondary input position.
4. Start the run.

### Isolation of DNA from Buccal Swabs

**Prepare samples**

- Perform the buccal scrape by following your standard laboratory procedure.

**Lyse buccal swab cells**

1. Swirl the swab in a 2-mL microcentrifuge tube containing 800 µL of **BloodPrep DNA Purification Solution** for 30 sec to 1.0 min.

   Note: If necessary, gently heat **BloodPrep DNA Purification Solution** to 37 °C for 5–10 min to dissolve precipitated salts.
2. Remove the swab, retaining as much liquid as possible in the microcentrifuge tube.
3. Pipette swab samples into the appropriate 96-well plate.
4. *Optional*.Store the lysed samples at 4 °C. If crystallization of salts is observed after storage, gently heat these samples for 5–10 min to 37 °C and then gently vortex.

**Perform purification run**

1. Set up the appropriate purification protocol for the 6700 Workstation.
2. Pipette the bucall lysate samples into a deep-well plate.
3. Place plate at the secondary input position.
4. Start the run.

### Reagents and Consumables

| Item | Part Number | Item | Part Number | Item | Part Number |
|---|---|---|---|---|---|
| 96-Well Deep-well plate | 4308641 | BloodPrep™ DNA Purification Solution | 4342775 | Proteinase K Solution (20 mg/mL) | 4333793 |
| 96-Well Optical Reaction Plate with Barcode | 4306737 | BloodPrep™ DNA Wash Solution | 4342949 | Splash Guard | 4311758 |
| Archive Tray Covers | 4306286 | BloodPrep™ PK Digestion Buffer | 4342777 | 300-µL, flat-bottom, 96-well cell culture plate | MLS[a] |
| BloodPrep™ DNA Elution Solution 1 | 4342951 | Genomic DNA Purification Tray II | 4330172 | Ethanol, 70% | MLS |
| BloodPrep™ DNA Elution Solution 2 | 4342950 | Microcentrifuge tubes, 2 mL | 4305936 | Water, molecular biology grade | MLS |

a.Major Laboratory Supplier

Applied Biosystems

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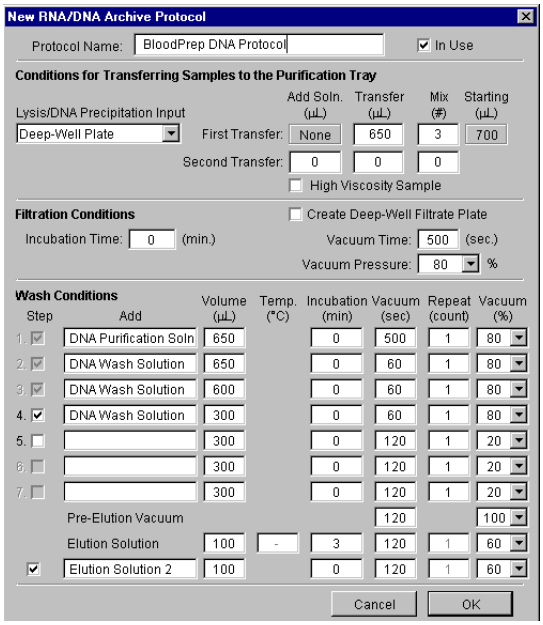
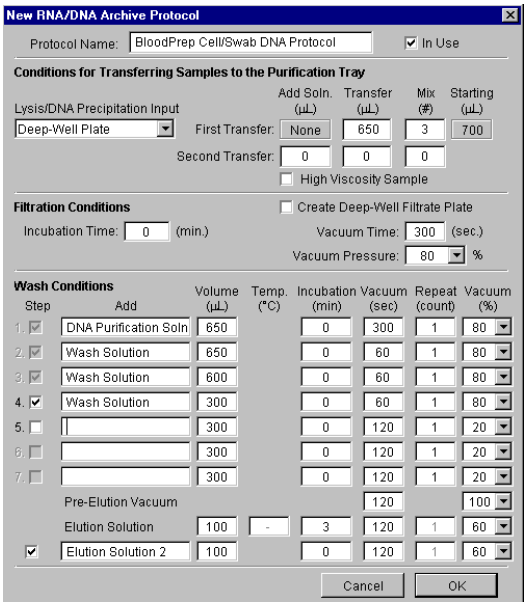
## DNA Isolation from Fresh and Frozen Blood, Tissue Culture Cells, and Buccal Swabs on the ABI PRISM™ 6700 Nucleic Acid Workstation *(continued)*

**⚠ WARNING** Biological samples have the potential to transmit infectious disease. For safety and biohazard guidelines, please refer to the "Safety" section in the *DNA Isolation from Fresh and Frozen Blood, Tissue Culture Cells, and Buccal Swabs Protocol*, P/N 4343586. Follow specific safety practices when using this instrument. For all chemicals in **bold** type below, please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

| STEP | ACTION | Lysate Preparation from Fresh or Frozen Whole Blood |
|---|---|---|
| 1 | Prepare samples | a. Pipette 15 µL of **Proteinase K Solution** (20 mg/mL) in 2-mL microcentrifuge tubes or deep well plates.<br>Note: If blood is <24 hrs old, add the samples directly to tubes with **Proteinase K Solution** and proceed to step 2b.<br>b. Add 85 µL of **PK Digestion buffer.**<br>c. Add 150 µL of blood to the digestion buffer and Proteinase K mixture.<br>Note: If using animal blood, use suggested volumes in Protocol, P/N 4343586.<br>d. Mix thoroughly by pipetting up and down 3 times. |
| 2 | Lyse whole blood | a. Incubate mixture at 58 °C for 10 min.<br>IMPORTANT! Temperatures in excess of 60 °C may cause degradation of isolated DNA and reduce activity of the Proteinase K.<br>b. Add 500 µL of **BloodPrep DNA Purification Solution** to generate a total of 750 µL.<br>IMPORTANT! The **BloodPrep DNA Purification Solution** may require gentle heating to 37 °C for 5–10 min to dissolve precipitated salts.<br>c. Mix the blood and **BloodPrep DNA Purification Solution** thoroughly by pipetting up and down 5 times.<br>IMPORTANT! The incubated blood sample mixture and purification solution must be thoroughly mixed with the pipette before proceeding, because vortexing does not effectively mix the viscous samples and the **Proteinase K Solution**, no matter how long you vortex.<br>d. *Optional*. If you cannot proceed with purification immediately, store the lysed samples at 4 °C. If crystallization of salts is observed after storage, gently heat these samples for 5–10 min to 37 °C and then gently vortex. |
| **STEP** | **ACTION** | **Lysate Preparation from Tissue Culture Cells** |
| 1 | Prepare samples | Remove the cells from the tissue culture media.<br>• For adherent cells, remove the tissue culture media from all wells by aspiration.<br>• For suspension cells, pellet the media by centrifugation and remove the supernatant by aspiration.<br>Note: For plasma, serum, and cerebrospinal fluid samples, pellet the cells by centrifugation following your standard laboratory procedures, and remove the supernatant by aspiration. |
| 2 | Lyse cells | a. Add 200–650 µL of **BloodPrep DNA Purification Solution** into each well of the appropriate 96-well plate.<br>Note: Do not exceed 650 µL in volume unless the sample is extremely viscous. If viscous, reduce the sample input volume or increase the volume of **BloodPrep DNA Purification Solution** appropriately.<br>IMPORTANT! The **BloodPrep DNA Purification Solution** may require gentle heating to 37 °C for 5–10 min to dissolve precipitated salts.<br>b. Mix the cells and **BloodPrep DNA Purification Solution** thoroughly by vortexing for 20 sec to 1.0 min, or by pumping the mixture with a pipette at least 5 times.<br>c. *Optional*. If you cannot proceed with purification immediately, store the lysed samples at 4 °C. If crystallization of salts is observed after storage, gently heat these samples for 5–10 min to 37 °C and then gently vortex. |
| **STEP** | **ACTION** | **Lysate Preparation from Buccal Swab Cells** |
| 1 | Prepare samples | Perform the buccal scrape by following your standard laboratory procedure. |
| 2 | Lyse cells | a. Swirl the swab in a 2-mL microcentrifuge tube containing 800 µL of **BloodPrep DNA Purification Solution** for 30 sec to 1.0 min.<br>IMPORTANT! The **BloodPrep DNA Purification Solution** may require gentle heating to 37 °C for 5–10 min to dissolve precipitated salts.<br>b. Remove the swab, retaining as much liquid as possible in the microcentrifuge tube.<br>c. Pipette swab samples into the appropriate 96-well plate.<br>d. *Optional*. If you cannot proceed with purification immediately, store the lysed samples at 4 °C. If crystallization of salts is observed after storage, gently heat these samples for 5–10 min to 37 °C and then gently vortex. |

| STEP | ACTION | Total DNA Purification of Blood, Tissue Culture Cells, or Buccal Cells Using the 6700 Workstation |
|---|---|---|
| **1** | Set up the 6700 Workstation | Set up the appropriate DNA purification parameters for your sample on the protocol tab of the 6700 Workstation software.<br>**⚠ WARNING** Read the MSDSs for **BloodPrep DNA Purification Solution, BloodPrep DNA Wash Solution,** and **BloodPrep DNA Elution Solution 1** and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.<br>**Fresh or Frozen Whole Blood Parameters for the 6700 Workstation**<br>  <br>**Tissue Culture Cell or Buccal Swab Cell Parameters**<br>  |
| **2** | Perform the purification run | a. Pipette the lysate samples into a deep-well plate.<br>b. Place the plate at the secondary input position.<br>Note: The maximum volume of lysate that the total DNA purification tray can accommodate is 600 µL.<br>c. Start the run. |

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