BloodPrep™ Chemistry

Quick Reference Card

DNA Isolation from Fresh and Frozen Blood, Tissue Culture Cells, and Buccal Swabs on the ABI PRISM™ 6100 Nucleic Acid PrepStation

⚠ 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 6100 Workstation.
2. Pipette blood lysate samples into purification tray.
3. 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 6100 PrepStation.
2. Pipette tissue lysate samples into purification tray.
3. 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 6100 Workstation.
2. Pipette buccal lysate samples into purification tray.
3. 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™ 6100 Nucleic Acid PrepStation *(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) into 2-mL microcentrifuge tubes or deep well plates. Note: If blood is <24 hrs old, add the samples directly to tubes with **Proteinase K Solution** and proceed to step 2b. b. Add 85 µL of **PK Digestion buffer.** c. Add 150 µL of blood to the digestion buffer and Proteinase K mixture. Note: If using animal blood, use suggested volumes in Protocol, P/N 4343586. d. Mix thoroughly by pipetting up and down 3 times.
2	Lyse whole blood	a. Incubate mixture at 58 °C for 10 min. IMPORTANT! Temperatures in excess of 60 °C may cause degradation of isolated DNA and reduce activity of the Proteinase K. b. Add 500 µL of **BloodPrep DNA Purification Solution** to generate a total of 750 µL. IMPORTANT! The **BloodPrep DNA Purification Solution** may require gentle heating to 37 °C for 5–10 min to dissolve precipitated salts. c. Mix the blood and **BloodPrep DNA Purification Solution** thoroughly by pipetting up and down 5 times. 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. 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. • 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. 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. 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. IMPORTANT! The **BloodPrep DNA Purification Solution** may require gentle heating to 37 °C for 5–10 min to dissolve precipitated salts. 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. 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. IMPORTANT! The **BloodPrep DNA Purification Solution** may require gentle heating to 37 °C for 5–10 min to dissolve precipitated salts. b. Remove the swab, retaining as much liquid as possible in the microcentrifuge tube. c. Pipette swab samples into the purification tray. 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 1 — Set up the 6100 PrepStation for blood samples

Total DNA Purification of Blood, Tissue Culture Cells, or Buccal Cells Using the 6100 PrepStation

Set up the DNA purification parameters for the blood sample on the protocol tab of the 6100 PrepStation software:

Step	Description	Volume (µL)	Position	Incubation (sec)	Vacuum (%)	Time (sec)
Note: Tape over empty wells of the purification tray with adhesive tape or an adhesive tray cover, or pre-wet all empty wells with 50 µL of **BloodPrep DNA Purification Solution** to ensure even vacuum. Position the carriage with the purification tray over the 6100 waste station.						
1	Load Samples[a]	650	Waste	0	80	300
2	Add **BloodPrep DNA Purification Solution**[a]	650	Waste	0	80	400
3	Add **BloodPrep DNA Wash Solution**	650	Waste	0	80	60
4	Add **BloodPrep DNA Wash Solution**	600	Waste	0	80	60
5	Add **BloodPrep DNA Wash Solution**	300	Waste	0	80	60
6	Pre-Elution Vacuum	–	Waste	0	100	120
7	Touch Off	–	Waste	–	–	–
8	**BloodPrep DNA Elution Solution 1**[b]	100	Collection	180	60	120
9	BloodPrep DNA Elution Solution 2[c]	100	Collection	0	60	120
10	Touch Off	–	Collection	–	–	–

a. If the well evacuates slowly, increase the vacuum setting to 100% and repeat the step.
b. Elution volume is between 75 µL and 200 µL in a standard PCR microplate. Elution volumes below 75 µL result in lowered yields of DNA. You may lower the total elution volume to 75 µL, but the volumes of **Elution Solution 1** and 2 must remain equal.
c. It is very important that you use the elution solutions in the correct order. **Elution Solution 1** must be incubated with the DNA on the membrane for 3 minutes to ensure maximum yield. Then an equal volume of Elution Solution 2 must follow to give the correct pH for DNA storage.

Set up the 6100 PrepStation for tissue culture or buccal swab samples

Set up the DNA purification parameters for the blood sample on the protocol tab of the 6100 PrepStation software:

Step	Description	Volume (µL)	Position	Incubation (sec)	Vacuum (%)	Time (sec)
Note: Tape over empty wells of the purification tray with adhesive tape or an adhesive tray cover, or pre-wet all empty wells with 50 µL of **BloodPrep DNA Purification Solution** to ensure even vacuum. Position the carriage with the purification tray over the waste station..						
1	Load Samples[a]	650	Waste	0	80	300
2	Add **BloodPrep DNA Purification Solution**	650	Waste	0	80	300
3	Add **BloodPrep DNA Wash Solution**	650	Waste	0	80	60
4	Add **BloodPrep DNA Wash Solution**	600	Waste	0	80	60
5	Add **BloodPrep DNA Wash Solution**	300	Waste	0	80	60
9	Pre-Elution Vacuum	–	Waste	0	100	120
10	Touch Off	–	Waste	–	–	–
11	**BloodPrep DNA Elution Solution 1**[b]	100	Collection	180	60	120
12	BloodPrep DNA Elution Solution 2[c]	100	Collection	0	60	120
13	Touch Off	–	Collection	–	–	–

a. For lysates with volumes in excess of 650 µL, use the Quick Run feature to pull 650 µL aliquots of lysate across the purification tray membrane, operating vacuum at 80% for 180 secs. Repeat until one aliquot remains to be added and then proceed to step 1 of the purification protocol. If the well starts to evacuate slowly after a number of additions, increase the vacuum setting to 100% and repeat the step.
b. Elution volume is between 75 µL and 200 µL in a standard PCR microplate. Elution volumes below 75 µL result in lowered yields of DNA. The total elution volume may be lowered to 75 µL, but the volumes of **Elution Solution 1** and 2 must remain equal.
c. It is very important that the elution solutions are used in the correct order. **Elution Solution 1** must be incubated with the DNA on the membrane for 3 minutes to ensure maximum yield. Then an equal volume of Elution Solution 2 must follow to give the correct pH for DNA storage.

STEP 2 — Perform the purification run

a. Pipette the lysate samples into the purification tray.
   Note: The maximum volume of lysate that the total DNA purification tray can accommodate is 600 µL.
b. Start the run.

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Printed in the USA. 05/2003

PN 4345297, Rev. A