TransPrep Chemistry

Chemistry on the ABI PRISM® 6700 Nucleic Acid Workstation

Quick Reference Card

⚠ WARNING Biological samples have the potential to transmit infectious disease. For safety and biohazard guidelines, please refer to the "Safety" section in the *TransPrep Chemistry Purification of gDNA from Filtrates Obtained After the Isolation of RNA from Homogenized Animal or Plant Tissue Samples Protocol*, P/N 4326965. 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 RNA and DNA

Animal and Plant Tissue

1. Remove tissue from organism.
2. Store tissue in **2X Nucleic Acid Purification Lysis Solution** at 4 °C.
3. *Optional*
   Freeze at –20 to –80 °C.

Run 1

Homogenize tissue in **1X lysis buffer** using one of the following:

- Rotor/Stator Homogenizer
- Bead Beater System

Refer to protocol P/N 4326965.

Optional

- Pre-filtration
- Digestion with **Proteinase K**

Preform purification on the 6700 Workstation.

1. Collect filtrates containing DNA in deep-well plates.
2. Retain deep-well plates for Run 2.

Run 2

Isolate DNA from tissue filtrates using TransPrep chemistry and reagents.

1. Add 400 µL **DNA Precipitation Solution** mixture to 200 µL filtrate retained from Run 1.
2. Load samples onto DNA Purification Tray 1.
3. Wash samples with **DNA Solutions I** and **2**.
4. Elute purified DNA.

Isolation of DNA directly

Animal and Plant Tissue

1. Remove tissue from organism.
2. Store tissue in **2X Nucleic Acid Purification Lysis Solution** at 4 °C.
3. *Optional*
   Freeze at –20 to –80 °C.

Homogenize tissue in **1X lysis buffer** using one of the following:

- Rotor/Stator Homogenize
- Bead Beater System

Refer to protocol P/N 4326965.

Optional

Pre-filtration

Bacteria

1. Isolate bacteria.
2. Store bacteria in **2X Nucleic Acid Purification Lysis Solution** at 4 °C.
3. *Optional*
   Freeze at –20 to –80 °C.

Incubate bacteria with one of the following:

- **Proteinase K** (65 °C, 15 min), vortex mix followed by addition of an equal volume of **2X Nucleic Acid Purification Lysis Solution**
- Lysozyme (37 °C, 1 h) followed by addition of an equal volume of **2X Nucleic Acid Purification Lysis Solution**
- Homogenize in a bead beater system in **1X lysis buffer**

Food Samples

1. Collect food sample.
2. Store sample in **2X Nucleic Acid Purification Lysis Solution** at 4 °C.
3. *Optional*
   Freeze at –20 to –80 °C.

1. Prepare sample.
   - Solid: Powder under liquid nitrogen and homogenize in **1X lysis buffer**.
   - Liquids: Add an equal volume of **2X Nucleic Acid Purification Lysis Solution** and homogenize.
   - Oils/Gels: Add an equal volume of **2X Nucleic Acid Purification Lysis Solution** and homogenize.
2. Incubate all samples at 100 °C, 12 min.

Pre-filter solid samples to remove particles using either

- Tissue Pre-Filter 1 or
- Centrifugation

Perform purification on 6700 Workstation.

1. Add 400 µL **DNA Precipitation Solution** mixture to 200 µL of sample from the above steps.
2. Load samples onto DNA Purification Tray.
3. Wash samples with **DNA Wash Solutions 1** and **2**.
4. Elute purified DNA.

Reagents and Consumables

RNA Purification	Part Number	gDNA Purification	Part Number
2X Nucleic Acid Purification Lysis Solution	4305895	DNA Precipitation Solution 1	4325962
RNA Purification Wash Solution 1	4305891	DNA Precipitation Solution 2	4325964
RNA Purification Wash Solution 2	4305890	DNA Wash Solution 1	4325958
Absolute RNA Wash Solution	4305545	DNA Wash Solution 2	4325960
Total RNA Purification Tray	4305673	DNA Elution Solution 1	4325956
Tissue Pre-Filter 1	4328129	gDNA Purification Tray 1	4318641
Deep-Well Plate	4308641		

Applied Biosystems

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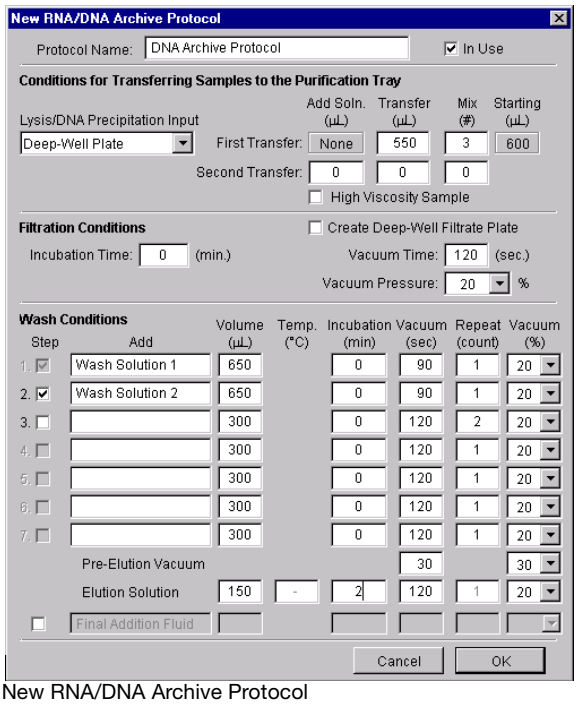
STEP	ACTION	
1	Remove and store tissue	a. Remove tissue from organism. b. Store tissue in **2X Nucleic Acid Purification Lysis Solution** or freeze at –20 to –80 °C. For other types of samples see the *TransPrep Chemistry Purification of gDNA from Filtrates Obtained After the Isolation of RNA from Homogenized Animal or Plant Tissue Samples Protocol.*
2	Prepare to perform Run 1	a. Create the following new protocols: • RNA Archive protocol, if performing Run 1 • DNA Precipitation protocol • DNA Archive protocol b. Lyse and homogenize the tissue. c. Perform pre-filtration, if the tissue sample needs it. d. Pipette 250 µL of each lysed and homogenized tissue sample into wells of a 300-µL flat-bottom cell-culture plate.  New RNA/DNA Archive Protocol
3	Perform Run 1	Perform Run 1 if RNA is required. Go to step 4 if only DNA is required. a. Make selections on the protocol tab. b. Set up the deckspace. c. Start the run. d. Finish the run.
4	Prepare to perform Run 2	a. Prepare **DNA Precipitation solution mixture**. Pipette the components listed below into a 120-mL reagent reservoir. IMPORTANT! These reagents should be mixed immediately before use and should not be stored as a mixture. **Component** / **Volume for One Well (µL)*** / **Volume for 96 Wells (mL)*** **DNA Precipitation Solution 1** / 100 / 9.6 **DNA Precipitation Solution 2** / 300 / 28.8 **Total Volume** / 400 / 38.4 * Volumes given are for 200 µL of filtrate per well collected in a deep-well plate during the first run on the 6700 Workstation. b. Load the reagent reservoir on the 6700 Workstation. c. Remove the deep-well plate from Run 1 and place the plate in the secondary input station.
5	Perform Run 2	a. Run both the new DNA Precipitation protocol and the new DNA Archive protocol. b. Make selections on the protocol tab. c. Set up the deckspace. d. Start the run. e. Finish the run.