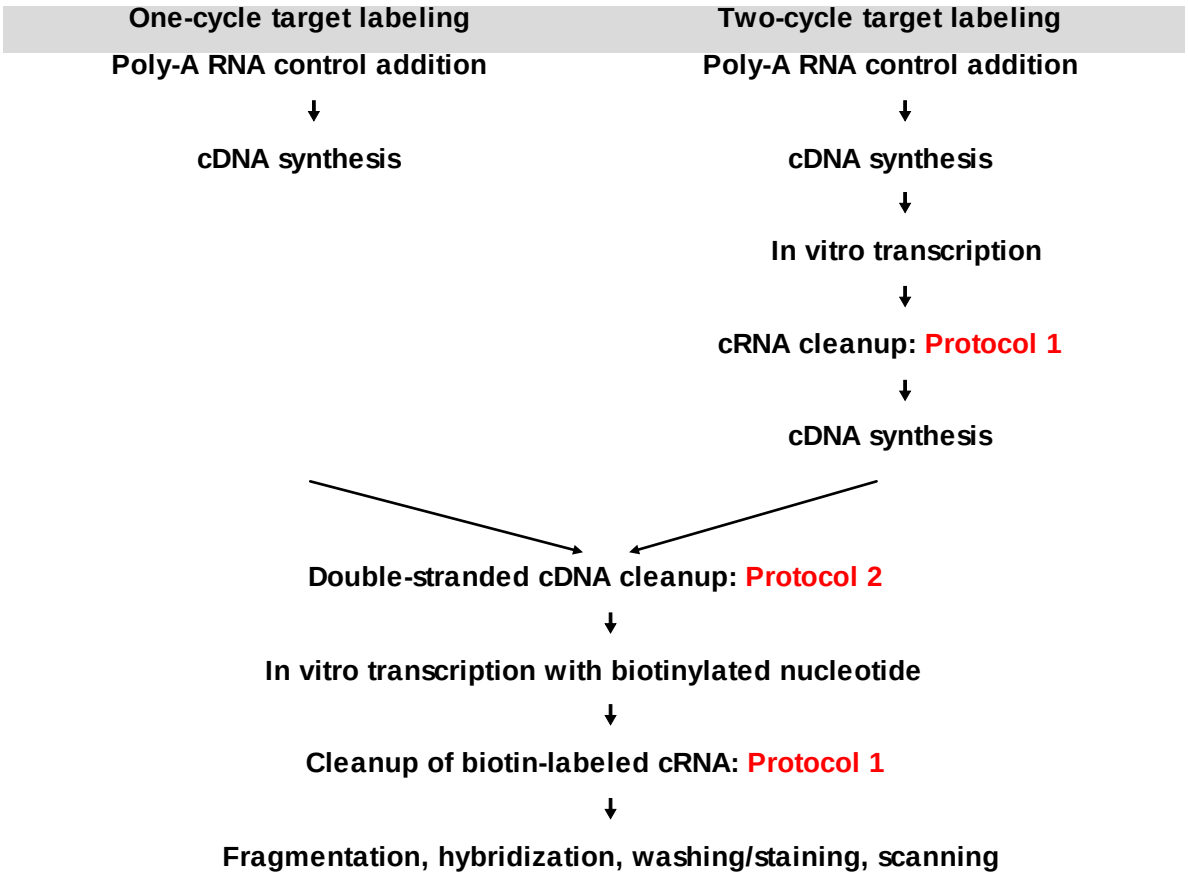
User-Developed Protocol

Sample cleanup for Affymetrix® GeneChip® expression probe arrays using the RNeasy® MinElute® Cleanup Kit or MinElute Reaction Cleanup Kit

These protocols have been adapted by customers from the RNeasy MinElute Cleanup Kit or MinElute Reaction Cleanup Kit protocols and are intended as a guideline for sample cleanup prior to analysis using Affymetrix GeneChip expression probe arrays. **These protocols have not been thoroughly tested and optimized by QIAGEN.**

Two protocols are provided. The first protocol, which uses the RNeasy MinElute Cleanup Kit, can be used for both cleanup of unlabeled cRNA after the first in vitro transcription in two-cycle target labeling and cleanup of biotin-labeled cRNA after in vitro transcription with biotinylated nucleotide in both one- and two-cycle target labeling. The second protocol, which uses the MinElute Reaction Cleanup Kit, describes cleanup of double-stranded cDNA before in vitro transcription with biotinylated nucleotide. The flowchart below details where protocols 1 and 2 are used in the Affymetrix GeneChip expression probe arrays workflow.

Affymetrix GeneChip expression probe arrays workflow



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IMPORTANT: Please read the "Safety Information" and "Important Notes" sections in the *RNeasy MinElute Cleanup Handbook* and/or the *MinElute Handbook* before beginning this procedure. For safety information on the additional chemicals mentioned in this protocol, please consult the appropriate material safety data sheets (MSDSs), available from the product supplier. The RNeasy MinElute Cleanup Kit and MinElute Reaction Cleanup Kit are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

Equipment and reagents to be supplied by user

- For cleanup of cRNA and biotin-labeled cRNA: RNeasy MinElute Cleanup Kit (cat. no. 74204)
- For cleanup of double-stranded cDNA: MinElute Reaction Cleanup Kit (cat. no. 28204 for 50 reactions or 28206 for 250 reactions)
- For cleanup of double-stranded cDNA: 3 M sodium acetate, pH 5.0, may be necessary
- Ethanol, 96–100% v/v
- For cleanup of cRNA and biotin-labeled cRNA: Ethanol, 80% v/v
- 1.5 ml or 2 ml microcentrifuge tubes
- Microcentrifuge

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Protocol 1: cRNA cleanup or biotin-labeled cRNA cleanup using the RNeasy MinElute Cleanup Kit

Important points before starting

- If using the RNeasy MinElute Cleanup Kit for the first time, read "Important Notes" in the *RNeasy MinElute Cleanup Handbook*.
- Buffer RLT contains a guanidine salt and is therefore not compatible with disinfecting reagents containing bleach. See the "Safety Information" section in the *RNeasy MinElute Cleanup Handbook*.
- All protocol and centrifugation steps should be performed at room temperature (15–25ºC). During the procedure, work quickly.
- This protocol is essential for biotin-labeled cRNA, as it removes unincorporated NTPs so that the concentration and purity of cRNA can be accurately determined by 260 nm absorbance.
- For biotin-labeled cRNA: save an aliquot of the unpurified in vitro transcription reaction for analysis by gel electrophoresis.

Things to do before starting

- Buffer RPE is supplied as a concentrate. Before using for the first time, add 20 ml ethanol (96–100%) as indicated on the bottle to obtain a working solution.
- Buffer RLT may form a precipitate during storage. If necessary, redissolve by warming, and then place at room temperature (15–25°C).

Procedure

1. **Adjust the in vitro transcription reaction to a volume of 100 µl by adding RNase-free water and mix by vortexing for 3 s.**
2. **Add 350 µl Buffer RLT to the diluted in vitro transcription reaction and mix by vortexing for 3 s.**
3. **Add 250 µl of 96–100% ethanol and mix well by pipetting. Do not centrifuge.**
4. **Transfer the sample (700 µl) to an RNeasy MinElute spin column placed in a 2 ml collection tube. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through and the collection tube.***
5. **Place the RNeasy MinElute spin column in a new 2 ml collection tube. Add 500 µl Buffer RPE to the spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through.**
   Reuse the collection tube in step 6.
6. **Add 500 µl of 80% ethanol to the RNeasy MinElute spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through.**
7. **Open the lid of the spin column, and centrifuge at full speed for 5 min. Discard the flow-through and collection tube.**

* Flow-through contains Buffer RLT and is therefore not compatible with bleach. See *RNeasy MinElute Cleanup Handbook* for safety information.

To avoid damage to their lids, place the spin columns into the centrifuge with at least one empty position between columns. Orient the lids so that they point in a direction opposite to the rotation of the rotor (e.g., if the rotor rotates clockwise, orient the lids counterclockwise). Centrifugation with the lids open ensures that no ethanol is carried over during elution.

8. **Perform elution as follows, depending on whether performing cleanup is of unlabeled cRNA or biotin-labeled cRNA.**

   For elution of unlabeled cRNA:
   Place the RNeasy MinElute spin column in a new 1.5 ml collection tube. Add 13 µl RNase-free water directly to the center of the spin column membrane. Close the lid gently, and centrifuge for 1 min at full speed to elute the RNA.

   The dead volume of the RNeasy MinElute spin column is 2 µl: elution with 13 µl RNase-free water results in an 11 µl eluate.

   For elution of biotin-labeled cRNA:
   Place the RNeasy MinElute spin column in a new 1.5 ml collection tube. Add 11 µl RNase-free water directly to the center of the spin column membrane. Close the lid gently, and centrifuge for 1 min at full speed to elute. Perform a second elution step by adding 10 µl RNase-free water to the center of the spin column membrane. Close the lid gently, and centrifuge for 1 min at full speed to elute.

   For subsequent photometric quantification of the purified cRNA, we recommend dilution of the eluate between 1:100 fold and 1:200 fold.

9. **Store cRNA at –20°C, or –70°C if not quantified immediately.**

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Protocol 2: Double-stranded cDNA cleanup using the MinElute Reaction Cleanup Kit

Important points before starting

- If using the MinElute Reaction Cleanup Kit for the first time, read "Important Notes" in the *MinElute Handbook*.
- Buffer ERC contains a guanidine salt and is therefore not compatible with disinfecting reagents containing bleach. See the "Safety Information" section in the *MinElute Handbook*.
- All protocol and centrifugation steps should be performed at room temperature (15–25ºC).

Things to do before starting

- Buffer PE is supplied as a concentrate. Before using for the first time, add ethanol (96–100%) as indicated on the bottle to obtain a working solution.
- If cDNA synthesis was performed in a reaction tube smaller than 1.5 ml, transfer the reaction mixture into a 1.5 ml or 2 ml microfuge tube (not supplied).

Procedure

1. **Add 600 µl Buffer ERC to the double-stranded cDNA synthesis reaction. Mix by vortexing for 3 s.**
2. **Check that the color of the mixture is yellow (similar to Buffer ERC without the double-stranded cDNA synthesis reaction).**

   If the color of the mixture is orange or violet, add 10 µl of 3 M sodium acetate, pH 5.0, and mix. The color of the mixture will turn yellow.
3. **Place a MinElute column in a 2 ml collection tube (supplied). Apply 500 µl mixture to the MinElute column and centrifuge for 1 min at ≥10,000 x *g*. Discard the flow-through.***

   Reuse the collection tube in step 4.
4. **Add the remaining mixture to the MinElute column and centrifuge for 1 min at ≥10,000 x *g*. Discard the flow-through and the collection tube.***
5. **Transfer the MinElute column to a new 2 ml collection tube (not supplied). Add 750 µl Buffer PE to the MinElute column and centrifuge for 1 min at ≥10,000 x *g*. Discard the flow-through.**

   Reuse the collection tube in step 6.
6. **Open the lid of the spin column, and centrifuge at full speed for 5 min. Discard the flow-through and collection tube.**

   To avoid damage to their lids, place the spin columns into the centrifuge with at least one empty position between columns. Orient the lids so that they point in a direction opposite to the rotation of the rotor (e.g., if the rotor rotates clockwise, orient the lids counterclockwise). Centrifugation with the lids open allows complete drying of the membrane.

* Flow-through contains Buffer ERC and is therefore not compatible with bleach. See *MinElute Handbook* for safety information.

7. **Place the MinElute column in a new 1.5 ml collection tube. Add 14 µl Buffer EB directly to the center of the spin column membrane. Close the lid gently. Incubate for 1 min at room temperature (15–25°C). Centrifuge for 1 min at full speed to elute the DNA.**

   Elution with 14 µl Buffer EB results in a 12 µl eluate.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.
Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from www.qiagen.com/Support/MSDS.aspx .

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