

Total RNA Preparation for Microarray Analysis

For preparation of microarray samples there are two methods of total RNA purification that are commonly used. Method I is well established and is commonly used by Affymetrix users. Method II, a shortened derivative of Method I, is the method recommended by Facility personnel. Note that the RNA Stat reagent may be substituted for Trizol in either of these protocols.

Method I. Purify the RNA using Trizol according to manufacturer recommendations. Take the resulting RNA and purify the RNA further using Qiagen RNeasy columns according to the RNA cleanup protocol. *NOTE: do not add BME to the Qiagen RLT buffer as this may increase GeneChip background.*

Method II. Trizol/RNeasy hybrid protocol. This method begins with Trizol homogenization and extraction. After this, the aqueous phase (containing the RNA) is combined with 70% EtOH and loaded onto an RNeasy column. Purification proceeds as described in Qiagen protocols. This method has the advantage of being simple and relatively short. It also limits handling of the RNA, perhaps reducing degradation.

Method II. Trizol/RNeasy hybrid protocol. Adapted from a communication by Alvydas Mikulskis (to the GENE-ARRAYS Mailing List) and the Qiagen RNeasy protocol (RNeasy Mini Handbook 05/99).

(Trizol portion)

1. Homogenize the starting material using an appropriate volume of Trizol. Store homogenate at RT for 5 min.

See manufacturer protocols for details regarding starting material, method of homogenization and Trizol volume.

2. Add chloroform to the homogenate (0.2 ml chloroform per ml of Trizol used) and shake vigorously for 15 seconds, then allow sample to sit at RT for 2-3 min.
3. Spin at 12,00x g for 15 min at 4°C.
4. Carefully remove aqueous phase (top) and transfer to new tube.

*It is **extremely important** not to get any of the material from the aqueous/organic interface; it is suggested to sacrifice aqueous material rather than risk taking this precipitate.*

5. Measure the volume of the aqueous sample.
6. **Slowly** add an equal volume of 70% EtOH, mixing as it is added.

Some precipitation may occur – this is OK.

(RNeasy portion)

7. Load the sample (up to 700 µl) into an RNeasy column seated in a collection tube. Be sure to include any precipitate that may have formed. Spin 30 sec at $\geq 8,000x g$. Discard the flow-through. If sample volume is larger than loading capacity of the column, repeat this step until the entire sample has been passed over the column.

If the expected yield is larger than the RNA-binding capacity of the column (100 µg for RNeasy), then the sample should be split and purified using multiple columns.

8. Add 700 µl buffer RW1 onto column and spin 30 sec at $\geq 8,000x g$.

9. Transfer column into a new collection tube, add 500 μ l Buffer RPE and spin 30 sec at \geq 8,000x g. Discard flow-through.

Make sure ethanol has been added to RPE.

10. Add 500 μ l Buffer RPE and spin 2 min at \geq 8,000x g. Discard flow-through.
11. Transfer column to new collection tube and spin (empty) for 1-2 min at \geq 8,000x g.
12. Transfer column into a new 1.5 ml collection tube and pipet 30-50 μ l of RNase-free water directly onto the column membrane. Allow the sample to sit at RT for 1-2 min, and then spin 1 min at \geq 8,000 x g to elute the RNA.

1) Incubation of the sample for 1-2 min prior to the spin is an optional step intended to improve yield

2) Because the subsequent enzymatic synthesis of cDNA from the RNA requires a high starting concentration of RNA (\geq 1 μ g/ μ l), it is suggested to use the minimum elution volume. If desired, the elution step can be repeated to attain any residual RNA from the column.

More information about total RNA preparation as well as the subsequent steps of sample preparation for microarray analysis can be obtained by downloading the "Sample Cleanup Module Protocol" at <http://www.affymetrix.com/products/reagents/specific/cleanup.affx>.