

Reagents and Materials Required

The following reagents and materials are recommendations and have been tested and evaluated by Affymetrix scientists. For supplier phone numbers in the U.S. and Europe, please refer to the Supplier and Reagent Reference List, [Appendix A](#) of this manual. Information and part numbers listed are based on U.S. catalog information. Additional reagents needed for the complete analysis are listed in the appropriate chapters. [Appendix A](#) contains a master list of all reagents used in this manual.

IMPORTANT

Do not store enzymes in a frost-free freezer.

Total RNA Isolation

- TRIzol Reagent, [Invitrogen Life Technologies](#), P/N 15596-018
- RNeasy Mini Kit, [QIAGEN](#), P/N 74104

Poly(A)⁺ mRNA Isolation

- Oligotex Direct mRNA Kit (isolation of mRNA from whole cells), [QIAGEN](#), P/N 72012, 72022, or 72041
- Oligotex mRNA Kit (isolation of mRNA from total RNA), [QIAGEN](#), P/N 70022, 70042, or 70061
- Qias shredder, [QIAGEN](#), P/N 79654 (Required only for use with QIAGEN Oligotex Direct Kit)
- DEPC-Treated Water, [Ambion](#), P/N 9920

cDNA Synthesis

- SuperScript II, [Invitrogen Life Technologies](#), P/N 18064-014 or SuperScript Choice System for cDNA Synthesis, [Invitrogen Life Technologies](#), P/N 18090-019

✓ Note

SuperScript Choice System contains, in addition to SuperScript II Reverse Transcriptase, other reagents for cDNA synthesis. However, not all components provided in the Choice System are used in the GeneChip cDNA synthesis protocol.

- T7–(dT)₂₄ Primer, [Genset Corp](#) or [Operon](#), HPLC purified DNA
5′ - GGCCAGTGAATTGTAATACGACTCACTATAGGGAGGCGG-(dT)₂₄ - 3′, 100 pmol/μL
- *E. coli* DNA Ligase, [Invitrogen Life Technologies](#), P/N 18052-019
- *E. coli* DNA Polymerase I, [Invitrogen Life Technologies](#), P/N 18010-025
- *E. coli* RNaseH, [Invitrogen Life Technologies](#), P/N 18021-071
- T4 DNA Polymerase, [Invitrogen Life Technologies](#), P/N 18005-025
- 5X Second strand buffer, [Invitrogen Life Technologies](#), P/N 10812-014
- 10 mM dNTP, [Invitrogen Life Technologies](#), P/N 18427-013
- GeneChip Sample Cleanup Module, [Affymetrix](#), P/N 900371

Synthesis of Biotin-Labeled cRNA



RNA Transcript Labeling Kit, [Affymetrix](#), P/N 900182

IVT cRNA Cleanup and Quantification

- GeneChip Sample Cleanup Module, [Affymetrix](#), P/N 900371
- 10X TBE, [BioWhittaker](#), P/N 16-012Y

cRNA Fragmentation

- GeneChip Sample Cleanup Module, [Affymetrix](#), P/N 900371

Miscellaneous Reagents

- Absolute ethanol (stored at -20°C for RNA precipitation; store ethanol at room temperature for use with the GeneChip Sample Cleanup Module)
- 80% ethanol (stored at -20°C; store ethanol at room temperature for use with the GeneChip Sample Cleanup Module)
- SYBR Green II, [BioWhittaker](#), P/N 50523; or [Molecular Probes](#), P/N S7586 (optional)
- Pellet Paint, [Novagen](#), P/N 69049-3 (optional)
- Glycogen, [Ambion](#), P/N 9510 (optional)
- 3 M Sodium Acetate (NaOAc), [Sigma-Aldrich](#), P/N S7899
- Ethidium Bromide, [Sigma-Aldrich](#), P/N E8751
- 1 N, NaOH
- 1 N, HCl
- 50 mM MgCl₂
- 0.5 M EDTA

Miscellaneous Supplies

- Sterile, RNase-free, microcentrifuge tubes, 1.5 mL, [USA Scientific](#), P/N 1415-2600 (or equivalent)
- Micropipettors, (P-2, P-20, P-200, P-1000), [Rainin Pipetman](#) or equivalent
- Sterile-barrier, RNase-free pipette tips (Tips must be pointed, not rounded, for efficient use with the probe arrays)
- Mini agarose gel electrophoresis unit with appropriate buffers
- Vacuum filter units (1 liter capacity, 0.22 µm or 0.45 µm), [VWR Scientific Products](#), P/N 28199-730
- UV spectrophotometer
- Heatblock
- Cooling waterbath