BECKMAN COULTER

Superior Recovery, Superior Data

AGENCOURT® RNACLEAN™

PURIFICATION FOR GENE EXPRESSION ANALYSIS

Agencourt RNAClean provides a simple, flexible and highly reproducible method for purifying nucleic acid products generated in common enzymatic reactions such as cDNA synthesis and *in vitro* transcription (IVT) reactions. This method utilizes Solid Phase Reversible Immobilization (SPRI®) magnetic bead-based technology. It is uniquely formatted for purification of both the cDNA and cRNA steps in Eberwine based procedures[1]. This technique is easily performed in manual or automated formats and eliminates the need for vacuum filtration or centrifugation. Agencourt RNAClean delivers superior nucleic acid recovery and purity for use in downstream microarray gene expression experiments.

Key Benefits

- Improved recovery requiring less starting material
- Compatible with sample clean-up for both the Affymetrix GeneChip One-Cycle Target Labeling kit and the Enzo Bioarray High Yield RNA transcript labeling kit.
- Same easy protocol for cDNA and cRNA purification
- Amenable to manual and automated processing
- Approved by Affymetrix for use in sample preparation for Affymetrix GeneChip® Array Station expression analysis.

High Yield and Consistent Recovery

Agencourt RNAClean consistently recovers more cRNA than standard column-based clean-up methods.

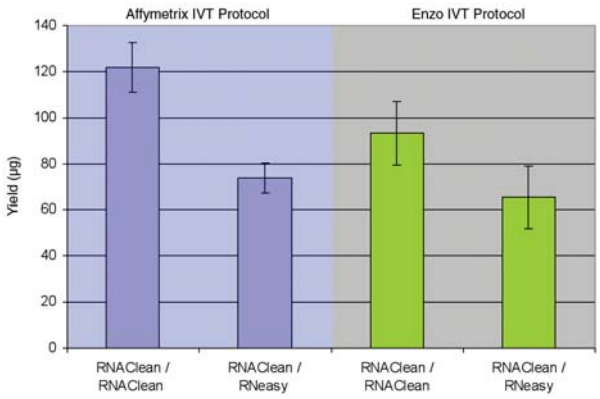

Figure 1. Comparison of cRNA Purification Methods.*
Agencourt RNAClean is effective in two popular methods for producing cRNA for microarray analysis. Five micrograms of rat brain RNA was used in cRNA labeling kits from Affymetrix and Enzo. IVT incubation time was 8 hours. Key: cDNA clean-up method/cRNA clean-up method.

It is compatible with both the GeneChip One-Cycle Target Labeling kit from Affymetrix and the Bioarray High Yield RNA transcript labeling kit from Enzo.

RNA Quality

For microarray experiments, it is important that the cRNA cleanup method be free of contaminating nucleases and that it isolates the full range of *in vitro* transcribed products with no bias toward recovery of smaller or larger products. Agencourt RNAClean is manufactured and tested to eliminate the introduction of RNase contaminants with the reagent. Agilent Bioanalyzer traces of cRNA purified with Agencourt RNAClean demonstrate that the full range of transcribed products is recovered (Figure 2).

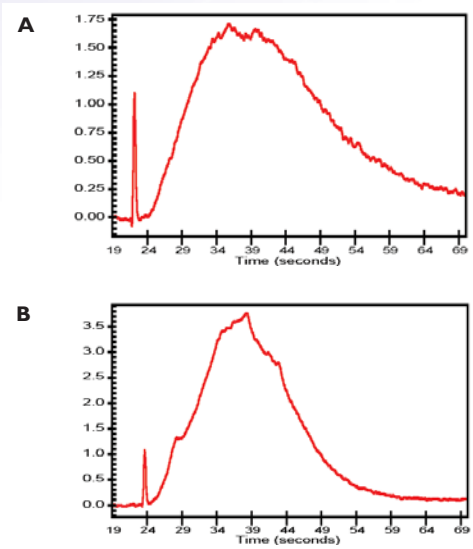

Figure 2. Comparison of Agencourt RNAClean to Column Cleanup.*
In vitro transcribed cRNA products produced from the same biological sample were purified using Agencourt RNAClean or Qiagen RNeasy columns. In this data provided by Gene Logic, both methods produce a typical profile. A) cRNA purified using Agencourt RNAClean. B) cRNA purified using Qiagen RNeasy columns.

Automation Friendly

Manually processing several samples in parallel is both time consuming and subject to inherent variability. Agencourt RNAClean provides a simple procedure for purification of *in vitro* reactions that is amenable to automation on many platforms including the Beckman Coulter Biomek® FX, NX, 3000, and ArrayPlex workstations (Figure 3).

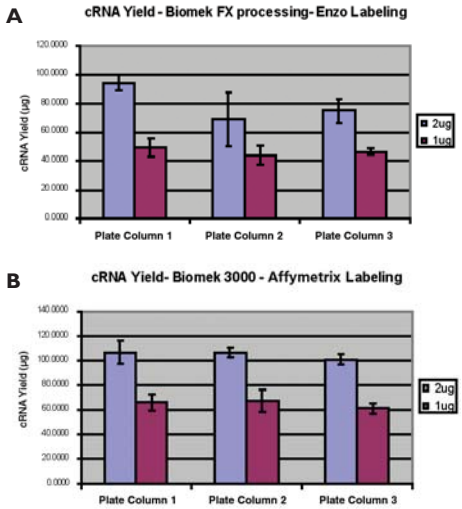

Figure 3. *Four technical replicates of HeLa total RNA (Ambion) were processed using A.) the Enzo cRNA labeling kit on the Biomek FX or B.) the Affymetrix labeling kit on the Biomek 3000 with 1 or 2 µg starting input total RNA.*

Data Correlation

Gene Expression data is not significantly altered when using Agencourt RNAClean as a sample clean-up method in RNA amplification protocols. Following extensive testing performed at Gene Logic, it was determined that Agencourt RNAClean does not have a statistically significant effect on expression metrics compared to standard processing as determined by Affymetrix Gene Chip analysis (Figure 4).

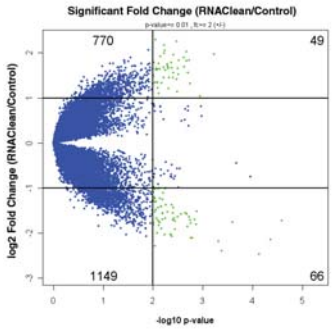

Figure 4. *There is no significant difference between expression profiles measured from samples processed with RNAClean versus sample processed with standard column based methods. Six human liver total RNA samples were amplified with Affymetrix One Cycle Target Labeling kit and cleaned up with both methods. The fold change in gene expression levels between the two methods were plotted against p-values. This plot demonstrates that only 151 fragments with greater than 2 fold change had a p value < 0.01. 547 are expected by chance.*

** Testing performed by Gene Logic, Inc.*

Summary

Agencourt RNAClean is a versatile technique that provides efficient recovery on a variety of testing platforms. It is a reliable and automation friendly method recovering high quality product without altering gene expression profiles. This method is compatible with multiple labeling technologies, allows for a unified protocol for cDNA and cRNA purification, and enables a reduction of input total RNA due to excellent recovery of cRNA.

References

1. Phillips J., and Eberwine J.H. 1996. Antisense RNA Amplification: A Linear Amplification Method for Analyzing the mRNA Population from Single Living Cells. Methods 10: 283–288.

Beckman Coulter Biomek FX

Ordering Information

For product pricing, please visit our website at **www.agencourt.com** or contact your local sales representative.

Product	Product #
Agencourt RNAClean 2 mL Tube Starter Kit	600561
Agencourt RNAClean 96-Well Plate Starter Kit	600560
Agencourt RNAClean 60 mL Bottle	000494

Related Products	Product #
Agencourt SPRIStand™, 6-Tube	001139
Agencourt SPRIPlate®, 96-Ring	000219
Agencourt RNAPrep™ Tube Starter Kit	001226
Agencourt RNAPrep 96-Well Starter Kit	001225

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