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Sample preparation using NuGEN Applause WT-Amp Plus ST System and Applause WT-Amp ST Systems for cDNA amplification for global expression analysis on Affymetrix GeneChip Gene 1.0 ST and Exon 1.0 ST arrays.

To ensure optimal performance of NuGEN's cDNA Amplification Systems, it is important to assess the quality of your RNA samples prior to planning your amplification. The Applause WT-Amp Plus ST system is designed to be used with high quality RNA samples.

RNA Isolation Methods

RNA samples must be free of contaminating proteins and other cellular material, organic solvents (including phenol and ethanol) and salts used in many RNA isolation methods. Use of a commercially available system for preparing small amounts of RNA that does not require organic solvents is recommended. If a method such as Trizol is used, choose high-quality Trizol and follow the isolation with an additional column purification.

One measure of RNA purity is the ratio of absorbance readings at 260 and 280 nm. Acceptable RNA purity is indicated by a A260:A280 in excess of 1.8. RNA samples with lower ratios may result in low amplification yield.

Purified total RNA samples of high molecular weight with little or no evidence of degradation are required for use with NuGEN Applause WT-Amp kits. Acceptable RNA integrity is indicated by a A260:A230 in excess of 1.8. RNA integrity can also be determined using the Agilent 2100 Bionalyzer. The RNA Integrity Number (RIN), available in the Bioanalyzer 2100 Expert software, provides an index of RNA quality that can be helpful in triaging purified RNA samples if varying integrity prior to amplification.

Recommended RNA Isolation Methods Include:

- Life Technologies Ambion RNA Later
- Trizol, followed by QIAGEN RNeasy
- Beckman Coulter Genomics Agencourt SPRI Bead Systems

DNase Treatment

The use of DNase-treatment is highly recommended when using purified RNA samples. Contaminating genomic DNA will interfere with accurate quantitation of RNA samples and may negatively impact detection sensitivity and data quality.

Positive Controls

The inclusion of a positive control RNA sample is recommended, as an essential tool in evaluating the success of an amplification experiment. Use a control RNA at an input level well above the minimum recommended input. This allows a performance baseline to be established.

We strongly recommend against the use of nucleic acid based carriers during RNA purification because many have been shown to produce cDNA product in first strand synthesis. We also advise against the use of glycogen in RNA isolation, as it inhibits reverse transcription.

Use RNase-Free Techniques

RNase contamination through reagents and work environment will lead to experimental failure. Follow these guidelines to minimize RNases in the workspace:

1. Wear disposable gloves and change them frequently.
2. Avoid touching surfaces or materials that could introduce RNases.
3. Use reagents provided. Substitutions may introduce RNases.
4. Clean work areas and instruments, including pipettes, with commercially available cleaning reagents, such as RNaseZap.
5. Use only new RNase-free pipette tips and microcentrifuge tubes.
6. Use a work area specifically designated for RNA work and do not use other high copy number materials in the same area.

RNA Storage

RNA samples should be stored at -80°C . Avoid frequent freeze/thaw cycles of RNA, as RNA degradation may result.

Amplified cDNA Storage

The amplified ST-cDNA produced by the Applause WT-Amp Plus ST System may be stored at -20°C .

Reference

The NuGEN Applause WT-Amp Plus ST System User Guide