

MICROARRAY FACILITY

Mount Sinai School of Medicine

Protocol #: D03 Version: 1 By: Te-Hua Chu, Ph.D, Date: 12/17/01 Page: 1 of 2

COMMENTS FOR RNA PREPARATION FOR MICROARRAY EXPERIMENT

Good quality of RNA is critical for microarray experiments. The preferred method for RNA extraction usually depends on the source of your samples. For a cell line, I'd prefer using Qiagen RNeasy kit. For a tissue sample, I'd prefer using Trizol reagent followed by RNeasy column. In some cases, additional RNA purification is required (detailed protocols are available on the website).

With Qiagen method, you will get higher purity but lower yield of RNA.

If your RNA source is rich of proteoglycans and/or polysaccharides (e.g. liver is rich of glycogen), I recommend you to further clean up your RNA as follows:

1. RNA suspension from Trizol method with or without precipitating with sodium citrate/NaCl solution.
 2. Add LiCl to the RNA solution as the final concentration to be 2.5 M.
 3. Incubate the tube at -20°C for 15 min.
 4. Spin the pellet at 5,000 rpm for 5 min.
 5. Wash 1X with 70% ethanol.
 6. Vortex the pellet until it completely disappears.
 7. Spin the pellet at full speed for 15 min.
 8. Resuspend the pellet in 300 μl of dH_2O .
 9. Precipitate RNA with sodium acetate.
 10. Wash the pellet 2X with 70% ethanol.
 11. Resuspend the pellet.
- Intensive washing is necessary. Trace of LiCl can interfere the subsequent reverse transcription reaction and results in low hybridization signal.

In order to take accurate OD 260/280 reading, RNA sample must diluted in 10mM Tris buffer (pH 7.0-8.0) at a small scale. Use a 50 μl cuvette with a minimum volume of 70 μl .