ACGT MICROARRAY FACILITY STANDARD OPERATING PROCEDURE		
TITLE: cDNA Synthesis and labelling		**PAGE: 1 of 2**
SOP#: MA005	**REVISION LEVEL: .1**	**DATE: 12 December 2005**
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1. **PURPOSE**
   This procedure describes RNA isolation from human cell lines.

2. **SCOPE**
   This protocol has been modified in order to obtain high quality RNA without proteins, which clog the membranes of the clean-up columns.

3. **MATERIALS**
   Qiazol/ TriReagent (Qiagen Scientific, catalogue number 79306 or Sigma; cat# T9424)
   RNeasy RNA isolation Kit (Qiagen; cat#74104)

4. **PREPARATION**

5. **PROCEDURE**

Harvest Cells
- Remove Medium
- Wash cells 2x with 5ml PBS

Disrupt Cells
- Add 1ml RLT buffer to flask (adding β-ME is preferable)
- Decant flask until all cells have lysed

Homogenize Cells
- Place QIAshredder into 2ml collection tube
- Pipette lysate into QIAshredder
- Centrifuge for 2min @ max speed (12000g)
- Pool flow through into large 50ml (Falcon) tube

Triagent Step
- Add 1ml Triagent/Qiazol for each collection tube used in step 3
- Store for 5min at room temperature
- Add 0.3ml chloroform for each collection tube used in step 3
- Cover the sample and shake vigorously
- Store @ room temperature for 2-15min

Centrifuge for 15 min @ 4°C @12000g
Remove the upper aqueous phase into a 50ml or 10ml tube

Precipitation

Add 1 Volume 70% ethanol
Mix well (Don't Centiguge)
Apply the mixture to the Qiagen columns (700µl per column)
Centrifuge for 15sec @ 10900rpm
Discard flow through

Wash Step 1

Add 350µl RW1 buffer to each column
Centrifuge for 15sec @ 10900rpm
Add DNase1 Mixture (70µl RDD buffer + 10µl DNase1)
NB!! Add to middle of column!!!
Store @ room Temp for 15min
Add another 350µl RW1 buffer to each column
Centrifuge for 15sec A10900rpm
Transfer to new sterile collection tubes

Wash Step 2

Add 500µl of RPE Buffer to each column
Centrifuge for 15sec @ 10900rpm
Discard the flow through
Transfer columns to new sterile collection tubes

Wash Step 3

Add another 500µl RPE buffer to each column
Centrifuge for 15sec @ 10900rpm
Transfer columns to new sterile collection tubes

Dry Step

Centifuge for 1min at full speed
Transfer the columns to new sterile eppies

Elution of RNA

Add 50µl of RNase-free water directly onto each column
Centrifuge for 1min at 10900rpm
The flow-through liquid contains the RNA
Repeat step to increase yield