RNA 003 v.009

Concentration of isolated RNA using Millipore Microcon Centrifugal DeviceYM-100

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1.0 Title

Concentration of Isolated RNA using Microcon Centrifugal Device YM-100

2.0 Purpose

To concentrate total RNA isolated from whole blood

3.0 Definitions and Abbreviations

SOP	Standard Operating Procedure
U.Pitt	University of Pittsburgh Genomics and Proteomics Core Facility

4.0 Equipment

	Name	Vendor	Catalog #
4.1.1	Microcon Centrifugal Device	Millipore	YM-100
4.1.2	Centrifuge	Sorvall Legend RT	
4.1.3	Spectrophotometer	Beckman	DU-800 DU-640
4.1.4	Rainin Micropipettes	Rainin	
4.1.5	1.5 ml Tubes .5 ml tubes	Fisher / PGC	

5.0 Main procedures

Note: These instructions should be used for the samples eluted in step 7.13 of the SOP RNA_ 02

5.1 Take out the samples prepared in SOP RNA_ 02

5.1.1 If the samples were previously frozen:
Take the samples out of the freezer and thaw on ice before concentrating
Then proceed to Step 5.2.

5.1.2 If you are using fresh sample recently eluted from ABI 6100 Prep-station then proceed to Step 5.2.

5.2 Preliminary Preparations

5.2.1 Label all tubes that you will use with the sample ID. Prepare 2 Microcon filter-tube sets for each sample.

5.2.2 Record the Microcon lot number in your work book.

5.2.3 Prepare Barcoded Labels and place on Stock Tubes (0.5 ml sterile tube with tethered cap PGC Scientific, P/N 16-8114-68). Refer to SOP RNA_001 section 5.4 for label setup. Use site code "-30" for these labels.

5.3 Insert 1 Microcon filter into each tube.
Two filter units are required for each sample.

5.4 Quick spin the samples, then:

- Transfer approximately 400 µl of Eluate on each of the two Microcon filters
- Keep Microcons in ice throughout the process
- Total volume of each sample is around 850 µl; the total volume of the sample that can be loaded on 1 Microcon is 500 µl

5.5 Spin the Microcons at 593 g (2,500 rpm) for 35 minutes at 4˚ C.

5.6 Take out the Microcons from the centrifuge and place them on a rack next to the corresponding clean 2.0 ml tubes.

5.7 Elution

5.7.1 From the 2 Microcons available for each sample elute 1 Microcon filter / at a time following the directions below.

5.7.2 Invert first filter into a clean, labeled tube for each patient sample and add 10 µl of nuclease free H_2O.

5.7.3 Spin at 1,163 g (3,500 rpm) for 5 minutes at 4˚ C.

5.7.4 After centrifugation, take second filter corresponding to the same sample and invert it into the same elution tube.

5.7.5 Add 10 µl of nuclease free H_2O to the filter.

5.7.6 Spin at 1,163 g (3,500 rpm) for 5 minutes at 4˚ C.

5.7.7 Now you have collected the entire sample into 1 elution tube per patient.

5.7.8 Take out 1 µl sample for spectrophotometer run.

5.7.9 Prepare RNA aliquots for the Agilent Bioanalyzer 2100 aliquots based on sample concentration from spectrophotometer using the scheme below:

- 2.0 µl sample for concentrations below 400 ng/ul
- 1.0 µl sample + 1.0 ul water for concentrations 400-1000 ng/ul
- 1.0 µl sample + 3.0 ul water for concentrations 1000 – 2000 ng/ul
- 1.0 µl sample + 5.0 ul water for concentrations above 2000 ng/ul

5.7.10 Transfer concentrated RNA into ITN Stock tubes. During transfer process verify the volume (ul) of the concentrate and document any variations in the Master Log.

5.7.11 Store concentrated RNA at -80° C.

5.8 Follow SOP RNA_04 for the instruction on how to run an Agilent Bioanalyzer 2100.

REVISION HISTORY			
Rev	**Section**	**Type**	**Initials/Dates**
001	Header/Footer	Modified the information contained in the header and footer	SK / 05.28.04
002	5.1	Wrong SOP listed. Changed to RNA_TEMPUS_001	SK / 05.28.04
003	4.1.2	Centrifuge type is modified to reflect the supplies on at the U. Pittsburgh site	SK / 05.28.04
004	5.5	Change in spinning time from 45 minutes to 35 minutes	SK / 05.28.04
005	5.7.3	Spinning time is modified from 3 minutes to 5 minutes	SK / 05.28.04
006	5.7.8	Insert: "Bioanalyzer aliquot is .5 to 1 µl based on concentration"	SK / 05.28.04
007	Front Page	Modify the ITN personnel responsible for signing and approval.	SK / 05.28.04
008	Front Page	Modify the ITN personnel responsible for signing and approval	SK / 07.29.04
009	5.0	Reference to section was corrected from 7.13.9 to 7.13	SK / 07.29.04
010	5.2.1	Change wording	SK / 07.29.04
011	5.7.8	Volume changed from max 1 µl to 2 µl	SK / 07.29.04
012	Appendix	Was taken out	SK / 07.29.04
013	All	Add the page numbers	SK / 09.30.04
014	5.4	Take out the 35ul aliquot to be used for a gel in the future	SK / 02.15.05
015	4.1.2	Change the type of the centrifuge	SK / 06.06.05
016	5.5 ; 5.7.3; 5.7.6	Change the centripetal force according to the modifications to the centrifuge type	SK / 06.06.05
017	5.7.8	Change the amount of the sample used in Bioanalyzer. Calculation is based on the Bioanalyzer sensitivity being between 50 – 500 ug/ul	SK / 09.09.05
018	All	Scheduled SOP review and update.	SK / 09.09.05
019	SOP number	SOP number has been updated from 002 to 03	SK / 09.09.05