RNA 002 v.008

Total RNA Isolation From Blood Collected into ABI Tempus™ Whole Blood Collection tubes

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1.0 Title

Total RNA Isolation From Blood Collected Into a ABI Tempus™ Whole Blood Collection tube

2.0 Purpose

To isolate total RNA from a blood sample collected into an ABI Tempus™ Tube

3.0 Definitions and Abbreviations

RT	Room Temperature
RNA	Ribonucleic Acid

4.0 Equipment and Reagents

4.1 Equipment

	Name	Vendor	Catalog #
4.1.1	Tempus Blood Collection tubes	Applied Biosystems	4342972
4.1.2	Centrifuge	Applied Biosystems	
4.1.3	20-ml Reservoir	Applied Biosystems	4344435
4.1.4	5-ml Reservoir	Applied Biosystems	4344437
4.1.5	Vortex Genie		
4.1.6	Large volume RNA prep filter	Applied Biosystems	4344439
4.1.7	2.0 ml microcentrifuge tubes	Applied Biosystems	4305936
4.1.8	Large volume adaptor plate	Applied Biosystems	4344438
4.1.9	ABI Prism 6100 Nucleic Acid Prep Station		
4.1.10	Spectrophotometer	Beckman	DU-800 DU-640
4.1.11	Agilent Bioanalyzer 2100	Agilent 2100	
4.1.12	2-ml tube collection plate	Applied Biosystems	4344436
4.1.13	Splash Guard	Applied Biosystems	4311758
4.1.14	5-ml ,10-ml, 25-ml pipettes	Fisher	
4.1.15	50-ml Falcon tubes	ISC Bioexpress	
4.1.16	5 ml syringes	Fisher	
4.1.17	Eppendorf Repeater	Fisher	

4.2 Reagents

	Name	Vendor	Catalog #
4.2.1	Absolute RNA Wash Solution	Applied Biosystems	4305545
4.2.2	Nucleic Acid Purification Elution Solution	Applied Biosystems	4305893

4.2.3	RNA Purification Wash Solution 1	Applied Biosystems	4305891
4.2.4	RNA Purification Was Solution 2	Applied Biosystems	4305891
4.2.5	70%Ethanol		

5.0 Blood Collection

Note: Blood will be collected at the clinical site and sent to the ITN repository located at Fisher BioServices. Blood then will be delivered to the ITN RNA isolation Core via FedEx overnight shipment.

5.1 Draw 3 ml blood directly into Tempus™ Blood RNA tubes per clinical site's procedures.

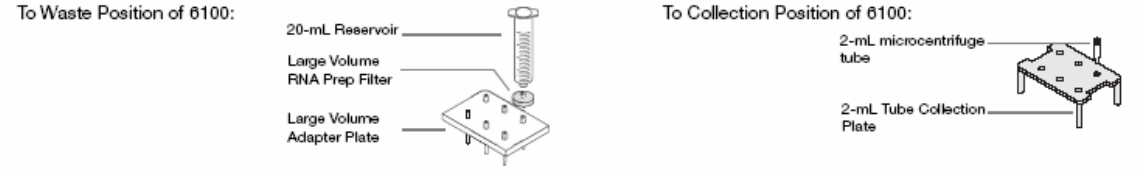
5.2 Immediately invert the tube at moderate pace for 10-20 seconds.

5.3 You can store the stabilized blood at 4˚ C up to 1 hour before putting it into a freezer.

5.4 Freeze down in –80˚ C freezer.

6.0 Assemble the Purification Parts for ABI Prism 6100 Prep-station

Assemble the purification consumable as shown in the following diagram:



6.1 Create a new method on the ABI 6100 machine. Enter all the values for the steps in Section 7.0 as listed in Appendix A.

6.2 Place a splashguard in the waste compartment.

6.3 Place assembled Large Volume Adapter Plate in the purification tray cartridge Secure the tray.

7.0 RNA Isolation

7.1 Allow blood to thaw completely.

7.2 While the blood is thawing, prepare the parts used in the extraction process by placing labels on each part that correspond to the ID's of the sample being extracted.

7.3 Record lot numbers of all materials used in the extraction process.

7.4 Using an Eppendorf Repeater, add 3 ml of 1X PBS to all 50 ml conical tubes.

7.5 Check every blood tube to make sure that it contains 3 ml of blood. Level of the solution in the tube should be at the black marker on the Tempus tube.
Each tube contains 6-ml of stabilizing reagent and 3 ml of blood.

7.5.1 1X PBS solution should be added to the tubes containing less then 9 ml total volume to make up for the missing volume of blood.

7.5.2 Please note in your lab book the volume of blood and 1X PBS solution added.

7.6 Transfer content of the Tempus™ tube into the 50 ml conical tube containing 1X PBS. Vortex on setting #7 for 30 seconds (timed).

7.7 Pre-wet the filter with 350 µl of RNA Purification Wash Solution 1.

7.7.1 Make sure that a highlighter on the machine is at "Step 1" position.

7.8 Load the sample on to the 20 ml Reservoir and vacuum at 80% in "Waste" position" until all lysate is through filter.

7.8 When vacuum stops, remove the 20 ml Reservoir and replace it with a 5 ml Reservoir.

7.9 In a new 5 ml Reservoir, add 4.5 ml of RNA Purification Wash Solution 1.

7.9.1 Vacuum at 80% in the "Waste" position. Make sure that there is no blood or other debris left on the filter. Step is complete when all lysate is through the filter.

7.10 Add 3.0 ml of Wash Solution 1 and apply 80% vacuum until all lysate is through the filter.

7.11 Add 3 ml of RNA Purification Wash Solution 2.
Vacuum at 80% for 120 seconds at "Waste" position.

7.12 Run the vacuum at 80% for 120 seconds to make sure that the filter is dry. When the vacuum stops, remove the reservoir.

7.12.1 The filter has to be completely Wash Solution 2 - free to prevent its interference in the removal of gDNA by AbsoluteRNA Wash Solution.

7.12.2 When the dry run is complete, remove the reservoir and replace it with a new 5 ml Reservoir.

7.13 DNAse Treatment

7.13.1 Add 350 µl of AbsoluteRNA Wash Solution and incubate for 900 seconds with 0% vacuum.

7.13.2 Add 3 ml of RNA Wash Solution 2 and incubate for 300 seconds.

7.13.3 Remove Wash Solution by applying 80% vacuum in "Waste" position.

7.13.4 Add 3 ml of RNA Wash Solution 2 and vacuum at 80% for 120 seconds in "Waste" position.

7.13.5 Repeat step 7.13.4

7.13.6 Apply 90% vacuum for 300 seconds.

7.14 Elution of RNA

7.14.1 Place 2-ml Collection Tubes (PGC Scientific P/N T339-6S) in the Tube Collection Tray in the collection compartment. Label tubes with appropriate sample ID labels.

7.14.2 Place filters with new 5-ml Reservoirs in a new Large Volume Adaptor Plate. Place Adapter Plate in position above the Tube Collection Tray.

7.14.3 Add 500 µl of Nucleic Acid Purification Elution Solution.

7.14.4 Incubate for 120 seconds at "Collection" position.

7.14.5 Apply 60% vacuum for 120 seconds in "Collection" position.

7.14.6 The eluate volume should be approximately 400 µl.

7.14.7 Add 500 µl of Nucleic Acid Purification Elution Solution and vacuum at 60% for 120 seconds in "Collection" position.
Total elution volume for each sample will be approximately 850 ul.

7.14.8 Please follow RNA_03 for Microcon Concentration and RNA_04 for Agilent Bioanalyzer 2100 QC process.
Keep RNA samples in ice if using right away, otherwise freeze at -80°C until used.

Appendix A

Please program the ABI 6100 Prep-station with the steps listed in a table below:
Times indicated in bold represent the complete number of seconds that are used. If the time is not bolded then vacuum is run until the sample goes through.

Step	Description	?	Volume	Position	Incubation (sec)	Vacuum Pressure	Time (sec)	Done?
1	Pre-wet – Wash Buffer 1		350 uL	Waste				
2	Apply Sample		13 mL	Waste		80%	500	
	Change reservoir – Use **5 mL reservoir**							
3	Wash 1 – Wash Buffer 1		4.5 mL	Waste		80%	500	
4	Wash 2 – Wash Buffer 1		3 mL	Waste		80%	500	
5	Wash 3 – Wash Buffer 2		3 mL	Waste		80%	120	
	Repeat Step 4 Vacuum			Waste		80%	**120**	
	Change reservoir – Use **5 mL reservoir**							
6	DNase Treatment – AbsoluteRNA		350 uL	Waste	900			
7	Wash 4 – Wash Buffer 2		3 mL	Waste	300	80%	120	
8	Wash 5 – Wash Buffer 2		3 mL	Waste		80%	120	
9	Wash 6 – Wash Buffer 2		3 mL	Waste		80%	120	
10	Pre-Elution Vacuum			Waste		90%	**300**	
11	Touch-Off			Waste				
12	Change Large Volume Adaptor Plate & reservoir (5 mL)							
13	Elution – Elution Buffer		500 uL	Collection	120	60%	**120**	
14	Elution – Elution Buffer		500 uL	Collection		60%	**120**	
15	Touch-Off			Collection				

Immune Tolerance Network

REVISION HISTORY:			
Rev	**Section**	**Type**	**Initials/Dates**
001	Front Page	Modified Header/Footer	SK / 05.28.04
002	4.0	Enter the sub-numbering	SK / 05.28.04
003	4.1	Add 4.1.16 and 4.1.17	SK / 05.28.04
004	4.1.15	Change vendor	SK / 05.28.04
005	5.0	Add a note about blood collection location	SK / 05.28.04
006	7.1	Change the thawing conditions for the blood samples to RT	SK / 05.28.04
007	7.3	Change the vendor name of the 50 ml Tube	SK / 05.28.04
008	7.4	Add clarification about the black line on the Tempus™ tube	SK / 05.28.04
009	7.5	Switch the order of steps 7.5 and 7.6	SK / 05.28.04
010	7.8	Take out 500 sec and change it to until the lysate is goes through the filter	SK / 05.28.04
011	7.9.1	Take out 500 sec. Step is completed when the solution goes through the filter	SK / 05.28.04
012	7.10	Add another wash with Wash Solution 1.	SK / 05.28.04
013	7.10.1	Change time from 150 sec to 120 sec	SK / 05.28.04
014	7.10.2	Take out this step. This was an extra wash step.	SK / 05.28.04
015	7.12.3	Take out the time. Run until the solution runs completely through the filter	SK / 05.28.04
016	7.13.6	List the related SOPs	SK / 05.28.04
017	Front Page	Name Change	SK / 07.15.04
018	7.4	Was inserted based on process flow	SK / 07.29.04
019	7.10	Wording	SK / 07.29.04
020	7.11	Time changed from 180 seconds to 120 seconds	SK / 07.29.04
021	Appendix	Modified table inserted	SK / 07.29.04
022	All	Add page numbers	SK / 09.30.04
023	7.10.1	Step was moved in to a separate step 7.11	SK / 06.06.05
024	All after 7.10	Change the Step numbering	SK / 06.06.05
025	All	Scheduled SOP review and update	SK / 09.09.05
026	SOP Name	SOP name change from RNA_TEMPUS001 to RNA_002	SK / 09.09.05