

Preparation of Peripheral Blood Mononuclear Cell Pellets and Plasma from a Single Blood Draw at Clinical Trial Sites for Biomarker Analysis

Paola Marco-Casanova¹, Natalia Lukashchuk¹, Benedetta Lombardi¹, Veerendra Munugalavadla², Melanie M. Frigault³, Elizabeth A. Harrington¹, J. Carl Barrett³, Andrew J. Pierce¹

¹Translational Medicine, R&D Oncology, AstraZeneca ²Acerta Pharma, AstraZeneca Group ³Translational Medicine, R&D Oncology, AstraZeneca

Corresponding Authors

Paola Marco-Casanova

paola.marco-

casanova@astrazeneca.com

Andrew J. Pierce

apierce@crecendobiologics.com

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Materials

Name	Company	Catalog Number	Comments
1.5 mL cryovial	Nalgene, ThermoFisher	5000-1020	To store PBMC pellets and re-suspended PBMCs
1.5 mL microcentrifuge tubes	VWR	525-0990	This is an example, use your preferred provider
15 mL conical sterile propylene centrifuge tube	Nunc, ThermoFisher	339651	Other brands can be used
2 mL screw cap tube sterile, with attached cap	ThermoFisher	3463	For plasma aliquoting
20X TBS Buffer	ThermoFisher Scientific	28358	Final is 25 mM Tris, 0.15 M NaCl; pH 7.5. This is an example, you can prepare your own stock or use a different provider
20X TBS Tween 20 Buffer	ThermoFisher Scientific	28360	Or supplement TBS with 0.05% to prepare TBST buffer
Automated cell counter or haemocytometer	ThermoScientific	AMQAX1000	We use Countess device and slides but could be other methods.
Adjustable micropipette allowing 50 µL measurements			To handle small volumes (i.e. western blot, transfer PBMC pellets to 1.5 mL tubes)
BD Vacutainer CPT mononuclear cell preparation tube (Na-citrate or Na-heparin) 8 mL	BD	362761, 362753	There are 4 mL tubes but if possible 8 mL tubes are recommended to obtain more PBMCs from a single blood draw
Cell-freezing box	ThermoFisher Scientific	5100-0001	This is an example, use your preferred provider.
Centrifugation unit converter	LabTools		http://www.labtools.us/centrifugation-speed-rpm-to-g-conversion/
DMSO	Sigma-Aldrich, MERK	D2438	Use your preferred provider. Used for PBMC cryopreservation

ECL horseradish peroxidase substrate	ThermoFisher	34075	Use your preferred reagent according to the sensitivity required to detect your biomarker by western blot. Other systems can be used such as IRDye secondary antibodies with imaging systems.
Faxitron MultiFocus X-ray cabinet	Faxitron Bioptics		To irradiate blood. Other models/makers are available
Fetal Bovine Serum (FBS), heat inactivated	ThermoScientific	102706	Use your preferred provider. Ued for PBMC cryopreservation
Fine tip, sterile 1.5 mL Pasteur pipettes	VWR	414004-018	Optional
Fixed-angle rotor centrifuge			Optional for preparation of plasma for ctDNA/metabolomics
Gel doc imaging system	SYNGENE		For imaging HRP developed membranes
Heat block			To denature lysates prior to run them in western blot, any maker equipped with suitable tube adaptors
Horizontal rotor (swing-out head) centrifuge	Thermoscientific	Heraeus Megafuge 40R	This is an example
Liquid nitrogen/dry ice			To flash-freeze samples
Marvel dried skimmed milk	Premier Foods		This is an example, use your preferred provider
Micropipette tips for range 1-200 µL			To handle small volumes (i.e. western blot, transfer PBMC pellets to 1.5 mL tubes)
NuPAGE 4-12% Bis-Tris protein gel, 1 mm, 10 wells	ThermoFisher Scientific	NP0321BOX	This is an example, cast your own or use your preferred provider
NuPAGE LDS Sample Buffer (4X)	ThermoFisher Scientific	NP0007	For imaging HRP developed membranes
NuPAGE Sample Reducing Agent (10X)	ThermoFisher Scientific	NP0009	This is an example.
PBS, no calcium, no magnesium	Gibco, ThermoFisher	14190-144	This is usually provided in the clinical kit.
Phosphatase inhibitor cocktail 2	Sigma-Aldrich, MERK	P5726-1ML	Optional for step 3.7
Phosphatase inhibitor cocktail 3	Sigma-Aldrich, MERK	P0044-1ML	Optional for step 3.7
Rabbit anti GAPDH	Cell Signaling Technology	CST 2128	1:1000 dilution in 5% milk TBST
Rabbit anti γH2AX	Cell Signaling Technology	CST 2577, lot 11	1:2000 dilution in 5 % milk TBST
Rabbit anti pS1981 ATM	Abcam	ab81292	1:2000 dilution in 5 % milk TBST
Rabbit anti pS635 RAD50	Cell Signaling Technology	CST 14223	1:1000 dilution in 5 % milk TBST
Rabbit anti total ATM	Abcam	ab32420	1:1000 dilution in 5 % milk TBST
Rabbit anti total RAD50	Cell Signaling Technology	CST 3427, lot 2	1:1000 dilution in 5 % milk TBST
RIPA buffer	Sigma-Aldrich, MERK	R0278-50ML	For cell lysis. This is an example, use your preferred provider
Sonicator	Diagenode	B01060010	Used for 3 cycles at 30 s on/ 30 s off, 4 oC. If using a different instrument, adjust number of cycles and intensity according to your sonicator.
Sterile 1.7 mL Pasteur pipettes	VWR	414004-030	This is an example, use your preferred provider
Sterile serological pipettes (5 and 10 mL volume)	Costar	4101, 4051	This is an example, use your preferred provider
Trypan blue	ThermoScientific	T10282	This is for the automated cell counter listed above.

Wet ice			To keep plasma samples and lysates cold
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