

Materials List for:

Production and Administration of Therapeutic Mesenchymal Stem/Stromal Cell (MSC) Spheroids Primed in 3-D Cultures Under Xeno-free Conditions

Joni H. Ylostalo¹, Nikolay Bazhanov², Arezoo Mohammadipoor³, Thomas J. Bartosh⁴

¹Department of Biology, University of Mary Hardin-Baylor

²Department of Pediatrics, University of Texas Medical Branch

³Multi-Organ Support Technology Task Area, U.S. Army Institute of Surgical Research

⁴Internal Medicine, Texas A&M University Health Science Center

Correspondence to: Thomas J. Bartosh at Bartosh@medicine.tamhsc.edu

URL: <https://www.jove.com/video/55126>

DOI: [doi:10.3791/55126](https://doi.org/10.3791/55126)

Materials

Name	Company	Catalog Number	Comments
MEM-α (minimal essential medium alpha)	ThermoFisher/Gibco	12561049; 12561056; 12561072	minimal essential medium for preparation of MSC growth medium (CCM)
FBS (fetal bovine serum), premium select	Atlanta Biologicals	S11595; S11510; S11550; S11595-24	component of complete culture media for all types of cells
L-glutamine	ThermoFisher/Gibco	25030081; 25030149; 25030164	component of complete culture media for all types of cells
Penicillin/Streptomycin	ThermoFisher/Gibco	15070063	component of complete culture media for all types of cells
Sterilization Filter Units, 0.22 μm PES membrane	MilliporeSigma	SCGPU01RE; SCGPU02RE; SCGPU05RE; SCGPU10RE; SCGPU11RE	media sterilization
150 mm cell culture dish	Nunc	D8554 SIGMA	cell culture
Thermo Forma water-jacketed CO ₂ humidified incubator	Thermo Fisher	Model 3110	incubation of cultured cells
Early passage MCSs	Center for the preparation and Distribution of Adult Stem Cells at The Texas A&M Health Science Center College of Medicine Institute for Regenerative Medicine at Scott & White	NA	preparation of 2D and 3D cultures of MSCs
water bath	VWR	89501-468	warming media to 37 °C
Pipettes	Eppendorf	492000904	manual liquid handling
Pipete-Aid	Drummond Scientific Company	4-000-300	handling serological pipettes
Costar sterile serological pipet (5, 10, 25 and 50 ml)	Corning	4487; 4101; 4251; 4490	liquid handling
PBS (phosphate buffered saline), pH 7.4	ThermoFisher/Gibco	10010023; 10010072; 10010031; 10010049	cell culture processing
0.25% trypsin/EDTA solution	ThermoFisher/Gibco	25200056; 25200072; 25200114	lifting adherent cells and dispersing cell aggregates
15 ml conical tube	Corning/BD Falcon	352097	cell centrifugation
50 ml conical tube	Corning/BD Falcon	352098	cell centrifugation
Eppendorf refrigerated centrifuge	Eppendorf/Fisher Scientific	Model 5810R	cell centrifugation
hemocytometer	Fisher Scientific	26716	cell counting
trypan blue	Sigma-Aldrich	T8154 SIGMA	dead cell exclusion during cell counting in hemocytometer
Defined xenofree MSC medium-1 (XFM-1)	ThermoFisher/Gibco	A1067501	Xeno-free media specifically formulated for the growth and

			expansion of human mesenchymal stem cells
Defined xenofree MSC medium-2 (XFM-2)	Stem Cell Technologies	5420	Defined, xeno-free medium for human mesenchymal stem cells
HSA (Human serum albumin)	Gemini	800-120	Component of xeno-free MSC media
rHSA (recombinant Human serum albumin)	Sigma-Aldrich	A9731 SIGMA	Component of xeno-free MSC media
Total RNA isolation Mini Kit	Qiagen	74104	Total RNA extraction
Qiashredder	Qiagen	79654	Sample homogenization prior to total RNA extraction
RNAse-free DNase Set	Qiagen	79254	On-column DNA elimination during total RNA extraction
β -mercaptoethanol	Sigma-Aldrich	M6250 ALDRICH	inhibition of RNases in RLT buffer
Vortex	VWR	97043-562	mixing sample
Spectrophotometer	Biorad	NA	RNA concentration and quality
High capacity cDNA Reverse Transcription Kit	ThermoFisher/Applied Biosystems	4368814	transcription of total RNA into cDNA
Gene Expression Assays	ThermoFisher/Applied Biosystems	varies	primer/probe combination for real-time PCR
Fast Universal PCR Master Mix	ThermoFisher/Applied Biosystems	4352042; 4364103; 4366073; 4367846	master mix for real-time PCR reaction
Real-time PCR system (ABI Prism 7900 HT Sequence Detection System)	ABI Prizm	NA	real-time PCR
1.5 ml centrifuge tube	Eppendorf	22364111	cell centrifugation, sample collection and storage
(-80 °C) freezer	Thermo Fisher	Model Thermo Forma 8695	sample storage
PGE2 (Prostaglandin E2) ELISA Kit	R&D Systems	KGE004B	estimation of cytokine concentration in the sample
DMEM (Dulbecco's modified Eagle medium)	ThermoFisher/Gibco	10566-016; 10566-024;10566-032	macrophage culture media
J774 mouse macrophages	ATCC	TIB-67	mouse macrophage cell line
12-well plate	Corning	3513	<i>in vitro</i> macrophage stimulation
LPS (lipopolysaccharide)	Sigma-aldrich	L4130	<i>in vitro</i> macrophage stimulation
Mouse TNF- α ELISA kit	R&D Systems	MTA00B	estimation of cytokine concentration in the sample
Mouse IL-10 (interleukin 10) ELISA kit	R&D Systems	M1000B	estimation of cytokine concentration in the sample
RPMLI-1640 medium	ThermoFisher/Gibco	11875-085	splenocyte culture media
BALB/c mice	The Jackson Laboratory	651	<i>in vivo</i> spheroid delivery; splenocyte preparation
Anti-Mouse CD3e Functional Grade Purified	eBioscience	145-2C11	<i>in vitro</i> splenocyte stimulation
70 μ m strainer	Corning	352350	Splenocyte preparation
Red blood cell lysis solution (1x)	Affymetrix eBioscience	00-4333	removal of red blood cells during splenocyte isolation
Mouse IFN- γ (interferon gamma) ELISA kit	R&D Systems	MIF 00	estimation of cytokine concentration in the sample
LNCaP prostate cancer cells	ATCC	CRL-1740	study the effect of 3D MSCs on cancer cell lines <i>in vitro</i>
DNA-based cell proliferation assay kit	ThermoFisher	C7026	cell number measurement based on DNA content

NaCl	Sigma-Aldrich	S5150	component of lysis reagent
EDTA (ethylenediaminetetraacetic acid)	ThermoFisher	FERR1021	calcium chelator, component of lysis reagent
Rnase A	Qiagen	19101	RNA degradation for measurement of DNA
Filter-based multi-mode microplate reader	BMG Technology	NA	Microplate assays (ELISA, cell quantification, <i>etc.</i>)
HBSS (Hanks balanced salt solution), no calcium, no magnesium, no phenol red	ThermoFisher/Gibco	14175079	resuspension of MSC spheroids prior to <i>in vivo</i> injections
Isoflurane	MWI Vet Supply	502017	Anesthesia for <i>in vivo</i> injections
Oxygen, compressed gas	Praxair	NA	For use with isoflurane
Thermo Forma BSL-2 cabinet	Thermo Fisher	Model 1385	Sterile cell culture
Safety I.V. catheter/needle stiletto, 20 G , 1 inch	Terumo	SR*FNP2025	Delivery of shperoids into peritoneal cavity
Sterile micropipette tips	Eppendorf	varies	liquid/cells handling