

Isolation and Culture of Bone Marrow-Derived Macrophages from Mice

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Materials

Name	Company	Catalog Number	Comments
20-cc syringe	DESCARPACK	SI100S4G	Sterile syringe
26-G needles	BD	497AQDKT7	Sterile needles
50-ml conical centrifuge tube	SARSTEDT	62547254	Plastic conical tubes suitable for centrifugation
70% ethanol solution	EMFAL	490	Ethanol solution for esterilization
Anatomical dissection forceps	3B SCIENTIFIC	W1670	Maintain in sterile beaker containing 70% ethanol solution
C57Bl/6 wild type mouse	Purchased from Biotério Central at Federal University of Minas Gerais	Not applicable	Mice must be specific-pathogen-free, age between 6 and 10 weeks. Mice need be accommodated at least one week earlier for recovering from the stress of transportation
Cell culture flask T175	GREINER	C7481-50EA	T-175 flask, canted neck, surface area 75 cm ² , with filter cap, DNase free, RNase free
Cell culture flask T75	GREINER	C7231-120EA	T-75 flask, canted neck, surface area 75 cm ² , with filter cap, DNase free, RNase free
Disinfecting or baby Wipes?	CLOROX	Not applicable	It helps cleaning the bone
Distilled Water	GIBCO	15230	Sterile distilled water
DMEM/F12-10	Not applicable	Not applicable	Add 10 mL of Fetal Bovine Serum (FBS) and 1 mL of Penicillin/Streptomycin (P/S) to DMEM/F12 (q.s.p. 100 mL)
DMEM/F12-10 + supernatant of L-929 cells	Not applicable	Not applicable	Add 20% of supernatant of L929 cells culture on DMEM/F-12-10
Dulbecco's Modified Eagle's Medium - F12 (DMEM/F12)	GIBCO	12500096	DMEM: F-12 Medium contains 2.5 mM L-glutamine, 15 mM HEPES, 0.5 mM sodium pyruvate, and 1200 mg/L sodium bicarbonate. Resuspend powder to 1 liter of distilled water and add 3,7 g of sodium bicarbonate. Adjust pH to 7,2

			and filter with 0,22 µM. Storage at 2 - 8 °C freezer
Fetal Bovine Serum, certified, heat inactivated, United States (FBS)	GIBCO	10082147	Enrichment for DMEM-F12
Hemocytometer	SIGMA-ALDRICH	Z359629	Used to count macrophages at microscopy
L-929 cells	SIGMA-ALDRICH	ATCC # CCL-1	L-929 is a lineage of mouse fibroblast cells used as a source of macrophage colony stimulating factor (M-CSF)
Nikon TI eclipse	NIKON	Not applicable	Nikon TI Eclipse is a fluorescence and phase contrast microscope
Non-enzymatic cell dissociation solution	CELLSTRIPER (CORNING)	25-056-CI	Non-enzymatic cell dissociation solution remove macrophages from the plate without damaging them
Non-treated round culture dishes 100 × 20–mm	CORNING	CLS430591	Do not use tissue culture treated petri dishes or any tissue culture treated plate
P3199 Penicillin G Potassium Salt	USBIOLOGICAL	113-98-4	Antibiotics
Penicillin and streptomycin (P/S) solution			Use 0,26 grams of penicillin and 0,40 grams of streptomycin. Mix with 40 mL of sterile PBS. Inside a horizontal laminar flow cabinet, use a 0,22 µM filter and store aliquots of 1.0 mL in 1.5 ml microfuge tubes in the freezer (-20°C)
Phosphate Buffered Saline free from Calcium and Magnesium (PBS)	MEDIATECH	21-040-CM	Sterile
S7975 Streptomycin Sulfate, 650-850U/mg	USBIOLOGICAL	3810-74-0	Antibiotics
Serological pipettes of 10mL or 25 mL	SARSTEDT	861254001	Serological pipettes is used volumes higher than 1 mL
Sodium Bicarbonate	SIGMA-ALDRICH	144-55-8	pH correction for DMEM-F12
Software Nis Elements Viewer	NIKON	Not applicable	NIS-Elements Viewer is a free standalone program to view image files and datasets
Sterile PBS and 2% of P/S solution	LABORCRIN	590338	Add 1 mL of P/S in 40 mL of sterile PBS
Straight iris scissors	KATENA	Not applicable	Maintain in sterile beaker containing 70% ethanol solution
Supernatant of L-929 cells	Not applicable	Not applicable	L-929 is a lineage of mouse fibroblast cells used as a source of macrophage colony stimulating factor (M-CSF). L-929 supernatant was obtain from the protocol
Surgical Scalpel Blade No.24 Stainless Steel	SWANN-MORTON	311	Used for exposing epiphysis from bones
Trypan Blue Solution, 0.4%	GIBCO	15250061	Trypan Blue Solution, 0.4%, is routinely used as a cell stain to assess cell viability using the dye exclusion test
Trypsin/EDTA solution 0,05%	GIBCO	25300-062	Used to dissociate cells from culture bottle
Water for Injection (WFI) for Cell Culture	GIBCO	A12873	Sterile and endotoxin-free water