

Materials List for:

Kupffer Cell Isolation for Nanoparticle Toxicity Testing

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Materials

Name	Company	Catalog Number	Comments
Euthatal (pentobarbital sodium)	Merial		
CD1 mice	Charles River	-	Mouse weight should vary from 35 to 45 g. We advise the use of male CD1 mice as their weight increase rapidly (e.g. a male CD1 mouse of 7 weeks old exceeds 35 g in weight). It is also advised to contact the animal supplier in advance to arrange for the delivery of older animals. Alternatively animals can be in-house to reach the desired weight.
HBSS (Ca ²⁺ and Mg ²⁺ free, with bicarbonate)	Life Technologies	14175-053	HBSS must be Ca ²⁺ free.
Ethylene Glycol Tetraacetic Acid (EGTA) tetrasodium salt	Sigma-Aldrich	E8145	EDTA can also be used but EGTA has the advantage of chelating Ca ²⁺ selectively.
HEPES (1 M)	Life Technologies	15630-056	100 ml
Collagenase type IV	Worthington	CSL-4	It is advised to test different batches of collagenase or at least mention to the supplier the product has to be suitable for liver cell isolation
Low glucose DMEM	Sigma-Aldrich	D5523	500 ml
RPMI (with sodium pyruvate and Glutamax)	Life Technologies	12633-012	500 ml
Penicillin/Steptomycin	Life Technologies	15140-122	100 ml
Fetal Bovine Serum	First-Link	60-00-850	500 ml
Trypan blue solution	Sigma-Aldrich	T8154	100 ml
Coated silica particle solution (Percoll)	GE Healthcare	17-0891-02	Percoll® is very stable and can be kept for several years
1x Phosphate Buffered Saline	Life Technologies	10010-023	500 ml
10x Phosphate Buffered Saline	Life Technologies	70011-036	500 ml
Dimethyl sulfoxide	Fisher	D/4120/PB08	500 ml
LDH kit (CytoTox 96)	Promega	G1781	Keep protected from light
DMEM phenol red free	Life Technologies	31053-028	500 ml
F4/80 antibody (Alexa 488)	AbD Serotec	MCA497A488	Do not dilute, used neat for flow cytometry
Fluorescent beads	Sigma	L2778	Latex beads, amine-modified polystyrene, fluorescent red. 1 ml
Name of the Material	Company	Catalog number	Comments/Description

Butterfly blood collection set (23 G/305 mm long tubing)	BD	367288	
Syringe Filters (0.22 µm Blue Rim)	Minisart	16534-K	
Centrifuge Tubes (50 ml Blue Cap)	BD Biosciences, Falcon	35 2070	
Petri dish (90 x 15 mm)	Thermo Fisher Scientific	BSN 101VR20	
100 µm cell strainer	BD	352360	
Peristaltic pump	Watson Marlow	SciQ 300	Rinse tubing before and after each usage with sterile PBS and 70% ethanol.
24-well plates	Corning	3526	
96-well plates	Corning	3595	
Centrifuge	Eppendorf	5810R	
Plate reader	BMG Labtech	FLUOstar Omega	
Serrefine forceps	Hammacher GmbH	Art. Nr. HSE 004-35 / Cat. Nr. 221-0051	The serrefine forceps allow to clamp the vessel cannulated with the 23 G needle without the need of holding the forceps during the perfusion procedure. URL: (http://www.hammacher.de/Laboratory-Products/Clamps-forceps/Serrefines/HSE-004-35-Serrefine::25126.html)
Flow cytometry tubes	BD Biosciences, Falcon	352052	
Microcentrifuge tubes (1.5 ml)	Elkay	000-MICR-150	
Cell scraper	BD Biosciences, Falcon	353086	Cut blade extremities with a pair of scissors to scrape cells in 24-well plates.
Name of the Reagent	Company	Catalog number	Comments/Description
EGTA (Ethylene Glycol Tetraacetic Acid)/HBSS (Hank's Balanced Salt Solution) Solution			HBSS containing 0.5 mM EGTA and 25 mM HEPES. Adjust pH to 7.4. Prepare 50 ml for each liver to perfuse.
Collagenase Solution			DMEM low glucose containing collagenase type IV at 100 UI/ml, 15 mM HEPES and 1% Penicillin/Streptomycin (v/v). Adjust pH to 7.4. Prepare 100 ml for each liver to perfuse. After adding the collagenase, it is advised to warm up the solution for 30 min before use. This allows the collagenase activity to be optimum.
Kupffer Cell Isolation Medium			RPMI containing, 1% Non-Essential Amino-Acids (v/v), 1% glutamax® (v/v) and 1% Penicillin/Streptomycin (v/v). Prepare at least 100 ml for 1-3 livers.
Kupffer Cell Culture Medium			RPMI containing 10% Fetal Bovine Serum, 1% Non-Essential Amino-Acids (v/v), 1% Glutamax® (v/v) and 1% Penicillin/Streptomycin (v/v). Prepare at least 100 ml for 1-3 livers.
SIP (solution of isotonic coated silica particles)			Mix 1.7 ml of 10x Phosphate Buffered Saline with 15.3 ml of Percoll® to obtain 17mL of SIP.
25% SIP solution			Mix 5 ml of SIP with 15 ml of 1x Phosphate Buffered Saline

50% SIP solution			Mix 10 ml of SIP with 10 ml of 1x Phosphate Buffered Saline
Lysis Buffer			DMEM media with 0.9% Triton X-100
PBS/BSA Solution			Prepare fresh Phosphate Buffered Saline pH 7.4 with 1% Bovine Serum Albumin.