

Materials List for:

A Microplate Assay to Assess Chemical Effects on RBL-2H3 Mast Cell Degranulation: Effects of Triclosan without Use of an Organic Solvent

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Materials

Name	Company	Catalog Number	Comments
RBL-2H3 Cells	ATCC	CRL-2256	The cells we used were a gift, but they are also available from ATCC
Triclosan/Irgasan	Sigma	72779 CAS# 3380-34-5	Should be stored in a low humidity environment
Trypsin	Gibco	25300-054 CAS# 3380-34-5	
EMEM	Lonza	12-611F	
Fetal Bovine Serum	Atlanta Biologicals	S11150	
Gentamycin Sulfate	Lonza Biological Sciences	17-518	
Albumin, Bovine Serum	Calbiochem	12659 CAS# 9048-46-8	
Surfact-Amps X-100 (Triton X-100; 10% solution)	Pierce	28314 CAS# 9002-93-1	
HEPES	J.T Baker	4153-01 CAS# 75277-39-3	
Magnesium Chloride	VWR	BDH0244-500G CAS# 7791-18-6	
D-(+)-Glucose	Biomedicals	152527 CAS# 50-99-7	
Potassium Chloride Crystal	J.T Baker	3046-01 CAS# 7447-40-7	
Calcium chloride dihydrate	Acros Organics	207780010 CAS# 10035-04-8	

Glycine	Sigma	G8898 CAS# 56-40-6	
4-Methylumbelliferyl-N-acetyl- β -D-glucosaminide (4-MU)	EMD Biosciences	474502-250MG CAS # 37067-30-4	Wrap in foil – is light-sensitive
Anti-DNP Mouse IgE	Sigma	D8406	Reagent has concentration of 1 mg/ml. Aliquot 25 μ l of reagent into separate microcentrifuge tubes and Parafilm. Store aliquots at -20 °C that are not being used and store aliquot that is being used at 2-8 °C for no longer than 1 month.
DNP-BSA	Gift from Dr. David Holowka and Dr. Barbara Baird, Cornell University		Suggest: life technologies DNP-BSA catalog# A23018
Calcium Ionophore A23187	Sigma	C75-22-1mg	Ionophore was made from a powder by adding 400 μ l of fresh 100% DMSO into the ionophore vial and is kept at -20 °C Note: we have used the ionophore past its 3 month expiration date successfully
DMSO	Sigma	D2650 CAS# 67-68-5	
Acetic Acid	VWR	BDH3094-2 CAS# 64-19-7	
Anhydrous Sodium Carbonate	Sigma	222321 CAS# 497-19-8	
Sodium Chloride	Sigma	71376 CAS# 7647-14-5	
Hydrochloric Acid	VWR	BDH3026 CAS# 7647-01-0	
Reference Buffer, pH 7	VWR	BDH5046	
Reference Buffer, pH 10	VWR	BDH5072	
Reference Buffer, pH 4	VWR	BDH5018	
pH electrode storage solution	VWR	14002-828	
Equipment:			
DU 7500 Spectrophotometer	Beckmann	No longer sold	
Synergy 2 plate reader Uses Gen5 Microplate Data Collection and Analysis Software	BioTek	Module S	

Hematocytometer	Hausser Scientific	3110	
7 x 7 CER HOT/STIR 120 V Combination hot plate/magnetic stir plate	VWR	97042-634	
Centrifuge	Eppendorf	5430	
Tissue culture water bath	VWR	Model# 89032-206	
Tissue Culture biological safety cabinet SafeGARD (TC hood)	The Baker Company	Model# SG403A-HE	
Tissue culture incubator	ThermoScientific	Model# 3598	
Pipetman	VWR		Range: P2-P1000
Balance	Mettler Toledo	Model# AG204	
pH meter	Symphony/VWR	Model# SB70P	
Pipet-Aid	Drummond Scientific	4-000-100	
Combitip dispenser	Eppendorf	4981 000.019	
Recipes:			
Acetate Buffer, pH 4.4	Make 0.12 M acetic acid and titrate to pH 4.4 with 10 N NaOH. This is 5.3 ml glacial acetic acid into 1 L of MilliQ water: $(1\text{ L}) \times (0.12\text{ mol/L}) \times (60\text{ g/mol}) \times (\text{ml}/1.37\text{ g}) = 5.3\text{ ml}$ because density of glacial is 1.37 g/ml	Sterile Filter into autoclaved glass bottle	
Substrate (4-MU)	Sigma M-2133, 250 mg, $\text{C}_{18}\text{H}_{21}\text{NO}_8$, FW 379.4 CAS (37067-30-4) Store in -20°C Stock: 0.12 M in DMSO (46 mg in 1 ml DMSO), warm to 37°C , vortex, sonicate 10 min. in water-bath sonicator with warm water, vortex again	For each experiment, make fresh solution of substrate in acetate buffer (100x dilution), for final concentration of 1.2 mM in acetate buffer	
Glycine Carbonate Buffer, pH 10	26.7 g glycine 47.1 g anhydrous sodium carbonate Add deionized water for 1 L, and adjust pH to 10	Sterile filter into autoclaved glass bottle	
Tyrodes (2 L), pH 7.4	135 mM NaCl: 15.78 g (or 270 ml of 1 M) 5 mM KCl: 10 ml of 1 M stock 1.8 mM CaCl_2 : 7.20 ml of 0.5 M stock 1 mM MgCl_2 : 4.00 ml of 0.5 M stock 5.6 mM glucose: 2.02 g (11.2 ml of 1 M) 20 mM HEPES: 40 ml of 1 M stock Using concentrated HCl pH from ~ 9.7 -7.4	Sterile filter into autoclaved glass bottle	
RBL Cell Media	Thaw fetal bovine serum (FBS, stored at -20°C) for about 4 hours in 37°C water bath Follow standard sterile technique Get out 1 L minimum essential medium (MEM) with L-glutamine (with Earle's salts) Pour off some MEM to have 800 ml MEM, add 200 mL	Sterile filter (0.2 mm) into autoclaved glass bottle	

	warm FBS Add 1 ml gentamicin sulfate antibiotic to 1 L of media with sterile pipette Only use media bottles that have been autoclaved and marked for cell culture use only.		
Plastic material used:			
200 µl Disposable sterile pipet tips with graduations in 96 rack	VWR	53509-009	polypropylene
1,000 µl Sterile aerosol pipet tips with HighRecovery	VWR	89003-420	polyethylene
10 µl micro tip low binding sterile	VWR	14217-704	polypropylene
Disposable/conical Microcentrifuge tubes for high G-force	VWR	20170-038	polypropylene
Disposable/graduated/conical/sterile 50 ml centrifuge tubes with screw caps	VWR	21008-178	polypropylene
Disposable/graduated/conical/sterile 15 ml centrifuge tubes with screw caps	VWR	21008-103	polypropylene
CELLSTAR Tissue Culture Treated T-25 Flask w/ Filter Cap	Greiner Bio One	690175	polystyrene
CELLSTAR Tissue Culture Treated T-75 Flask w/ Filter Cap	Greiner Bio One	658175	polystyrene
CELLSTAR 10 ml Paper/Plastic Wrapped Serological Pipette	Greiner Bio One	607180	polystyrene
CELLSTAR 2 ml Paper/Plastic Wrapped Serological Pipette	Greiner Bio One	710180	polystyrene
CELLSTAR 5 ml Paper/Plastic Wrapped Serological Pipette	Greiner Bio One	606180	polystyrene
CELLSTAR 25 ml Paper /Plastic Wrapped Serological Pipette	Greiner Bio One	760180	polystyrene
1 cm cuvettes	N/A	N/A	polystyrene
CELLSTAR, 96W Microplate, Tissue-Culture Treated, Black, with Lid 96-well Plate	Greiner Bio One	655086	polystyrene
Combitips	Eppendorf	022266501	Polypropylene/ polyethylene