

Materials List for:

A Rapid and Specific Microplate Assay for the Determination of Intra- and Extracellular Ascorbate in Cultured Cells

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Materials

Name	Company	Catalog Number	Comments
Nunc 96-well flat-bottom plates	Thermo	269620	Any flat-bottom 96-well plate can be used
Refrigerated benchtop microcentrifuge	Eppendorf	5415D	A non-refrigerated microcentrifuge that has been equilibrated to temperature in a cold room can also be used
Refrigerated bench-top centrifuge	Eppendorf	5810R	Swing-bucket
Bio-Rad Benchmark Plus Microplate Spectrophotometer	Bio-Rad		Any microplate spectrophotometer capable of reading at 593 nm can be used and is recommended. If a filter-based plate reader is used, choose the closest wavelength possible and use the standard-curve method.
Eppendorf MixMate (microplate orbital mixer)	Eppendorf		This is a very versatile and reliable microplate mixer and works very well for these assays
General-purpose buffers			
Phosphate-buffered saline (PBS), pH 7.4			
MOPS-buffered saline (MBS); 137 mM NaCl, 2.7 mM KCl, 15 mM MOPS-Na ⁺ , pH 7.3			
MBS + 5 mM D-glucose (MBS/D)			
HEPES-buffered saline + 5 mM D-glucose (HBS/D); 137 mM NaCl, 5.2 mM KCl, 1.8 mM CaCl ₂ ·2 H ₂ O, 0.8 mM MgSO ₄ ·7 H ₂ O, 5 mM D-glucose, 20 mM HEPES-Na ⁺ , pH 7.3)			
Cell permeabilisation buffer (CPB; 0.1% saponin in PBS)			
General chemicals			
L-ascorbic acid or sodium L-ascorbate	Sigma-Aldrich		Highest purity preparations should be obtained
Dehydro-L-ascorbic acid (DHA) dimer	Sigma-Aldrich	30790	Aqueous solutions theoretically yield 2 moles of DHA monomer per mole of DHA dimer
Cytochalasin B	Sigma-Aldrich	C6762	Stock solutions prepared in DMSO or ethanol

Ascorbate oxidase (AO)	Sigma-Aldrich	A0157	Stock solutions (120 U/ml) can be prepared in PBS or MBS and then frozen in aliquots
Potassium ferricyanide (FIC)	Sigma-Aldrich	455989	Trihydrate
Ferene-S (3-(2-Pyridyl)-5,6-di(2-furyl)-1,2,4-triazine-5',5''-disulfonic acid disodium salt)	Sigma-Aldrich	92940	
Sodium L-glutamate	Sigma-Aldrich		
L-glutamine	Sigma-Aldrich		
Saponin	Sigma-Aldrich	47036	Prepare a 0.1% stock solution
Stock solutions for intracellular ascorbate determination assay			
3 M sodium acetate (pH 6.0)			
Glacial acetic acid			
0.2 M citric acid			
3.3 mM FeCl ₃ in 0.1 M acetic acid			
30 mM ferene-S			
50% (v/v) acetic acid + 30% (w/v) trichloroacetic acid (TCA)			
Stock solutions for ascorbate-efflux assay			
AO (120 U/ml)			
2.4 mM ferene-S			
0.12 mM FeCl ₃ in 0.6 mM sodium-citrate			