

Materials List for:

Pulse-chase Analysis of N-linked Sugar Chains from Glycoproteins in Mammalian Cells

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Materials

Name	Company	Catalog Number	Comments
600E Multisolvant delivery System controller	Waters	WAT062710	
Acetonitrile LiChrosolv (gradient grade for liquid chromatography)	Merck & Co., Inc.	1.0003	
Microcon Amicon Ultra 0.5 ml 30K or Centricon ultracel YM-30	EMD Millipore	UFC503024 or 4208	
Concentrator 5301, incl. 48 x 1.5 / 2.0 ml fixed-angle rotor	Eppendorf	5301 000.016	
Dialyzed F-tal Calf Serum	Biological Industries	04-011-1A	
Dulbecco's Modified Eagle's Medium	GIBCO, by Life Technologies	41965039	
Dulbecco's Modified Eagle's Medium - glucose free	Sigma-Aldrich	D5030	
Dulbecco's Phosphate Buffered Saline (PBS)	Sigma-Aldrich	D1408	
EDTA disodium salt(Titriplex Iii)	Merck & Co., Inc.	1084180250	
Endo Hf Kit (1,000,000 units/ml)	New England Biolabs	p0703S	
F-tal Calf Serum (FCS)	Biological Industries	04-001-1A	
Frac-100 fraction collector	Amersham	18-1000-77	
LS 6500 Liquid Scintillation Counting systems	Beckman Coulter Inc.	510720	
Mannose, D-[2- ³ H(N)]- (Specific Activity: 15-30Ci/mmol)	PerkinElmer, Inc.	NET570A	
N-carbobenzoxyl-leuciny-leuciny-leucinal (MG-132)	Calbiochem	474790	
N-glycosidase F (1000 units/ml)	Roche Group	11365177	
N-octylglucoside	Sigma-Aldrich	O3757	
NIH 3T3 cells	American Type Culture Collection	CRL-1658	
Opti-Flour	PerkinElmer, Inc.	6013199	
Phosphoric acid solution (49-51%, for HPLC)	Fluka	79607	
Protease Inhibitor Cocktail	Sigma-Aldrich	P2714	
Protein A-Sepharose beads	Repligen	IPA300	
Rabbit polyclonal anti-H2 carboxy-terminal ⁶			
Sodium deoxycholate	Sigma-Aldrich	30970	
Sodium dodecyl sulfate	Bio-Rad	161-0301	
Sodium phosphate	Sigma-Aldrich	342483	

Sodium pyruvate solution (100mM)	Sigma-Aldrich	S8636	
Spherisorb NH2 Column, 5 µm, 4.6 x 250 mm	Waters	PSS831115	
Triton X-100	VWR	306324N	
Vibra-Cell ultrasonic processors VCX 750	Sonics and Materials, Inc.	690-003	
Buffer A: 1% Triton X-100, 0.5% w/v sodium deoxycholate, Protease inhibitor cocktail 2% v/v in PBS			
Buffer B: 0.5% w/v SDS, Protease inhibitor cocktail 2% v/v in PBS			
Buffer C: N-glycosidase F reaction buffer: 200mM Na ₃ PO ₄ , pH 8.0, 4mM EDTA, 1.5% N-octylglucoside			
NIH 3T3 stably expressing H2a ⁶			
Buffer D: 0.5% Triton X-100, 0.25% w/v sodium deoxycholate, 0.5% w/v SDS, Protease inhibitor cocktail 2% v/v in PBS			
Buffer E: 0.5% w/v SDS, 1% v/v 2-Mercapto-ethanol, Protease inhibitor cocktail 2% v/v in PBS			
2-mercapto-ethanol	Sigma-Aldrich	M3148	