

Materials List for:

Measuring TCR-pMHC Binding *In Situ* using a FRET-based Microscopy Assay

Markus Axmann¹, Gerhard J. Schütz¹, Johannes B. Huppa²

¹Institute for Applied Physics - Biophysics, Vienna University of Technology

²Institute for Hygiene and Applied Immunology, Medical University of Vienna

Correspondence to: Johannes B. Huppa at Johannes.Huppa@meduniwien.ac.at

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Materials

Name	Company	Catalog Number	Comments
LB-media	Fisher Scientific	10000713	bacterial expression
Sf900 II	Life Technologies	10227402	insect cell media for baculo virus production
Insect-XPRESS with L-glutamine (Lonza)	Fisher Scientific	10564038	insect cell media for baculo virus expression
Sf9 cells	Life Technologies	11496-015	cells for virus production and expansion
High Five Cells	Life Technologies	B855-02	cells for potein expression
LB-media	Fisher Scientific	10000713	bacterial expression
Centramate System	Pall		protein concentrartion from large volumes
Centramate cassette 10kDa cutoff	Pall	OS010T12	protein concentrartion from large volumes
Amicon Ultra-15 Centrifugal Filter Units	EMD Millipore	UFC900308	protein concentrartion
Amicon Ultra-4 Centrifugal Filter Units	EMD Millipore	UFC800308	protein concentrartion
Amicon Stirred Ultrafiltration Cell Model 200 mL	EMD Millipore	5123	protein concentrartion
Äkta pure 25L	GE Healthcare	29-0182-24	protein purification
Superdex 200 10/300 GL	GE Healthcare	17-5175-01	protein purification
Superdex 75 10/300 GL	GE Healthcare	17-5174-01	protein purification
Mono Q 5/50GL	GE Healthcare	17-5166-01	protein purification
Ni Sepharose 6 Fast Flow	GE Healthcare	17-5318-01	protein purification
Tricorn 10/20 column	GE Healthcare	28-4064-13	protein purification
Gilson HPLC system	Gilson		purificationof fluorochrome-coupled peptides
Pursuit XRs C18, 5 µm particle size, 21.2*250mm column size	Agilent	A6000250X212	purificationof fluorochrome-coupled peptides
Pursuit XRs C18, 5 µm particle size, 21.2*50 mm column size	Agilent	A6000050G212	purificationof fluorochrome-coupled peptides
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Cy3 maleimide	GE Healthcare	PA23031	site-specific protein labeling via mutant unpaired cysteines
Cy5 maleimide	GE Healthcare	PA25031	site-specific protein labeling via mutant unpaired cysteines
Alexa Fluor 555 C2 Maleimide	Life Technologies	A-20346	site-specific protein labeling via mutant unpaired cysteines
Alexa Fluor 647 C2 Maleimide	Life Technologies	A-20347	site-specific protein labeling via mutant unpaired cysteines
Fura-2, AM, cell permeant	Life Technologies	F-1221	calcium-sensitive dye for cell labeling
dimethyl sulfoxide	Sigma Aldrich	151874	for dissolving fura-2 am
Hank's Balanced Salt Solution plus calcium/magnesium	Fisher Scientific	10225362	imaging buffer
PBS	Life Technologies	14190-136	
Bovine Serum Albumin lyophilized powder	Sigma Aldrich	A2153	supplement for imaging buffer
14-4-4S antibody	affimetrix eBioscience	14-5980-81	blocking antibody for H2-I-Ek (recognized by the 5c.c7, 2B4 and AND TCR)
5 ml polypropylene round-bottom tube	Becton Dickinson	FALCON 352063	
0.22 µm Ultrafree-MC centrifugal filter unit	EMD Millipore	UFC30GV0S	
Syringe filter 0.2µm	Millipore	GVWP04700	
TetraSpeck Microspheres, 0.1 µm, fluorescent blue/green/orange/dark red	Life technologies	T-7279	
Microscope for fura-2-based calcium measurements	LEICA	DMI4000B	
Microscope for (single molecule) FRET measurements	LEICA/ZEISS/NIKON/OLYMPUS	for details please refer to parallel JoVE contribution by Axmann et al.	
planar supported lipid bilayers		for details please refer to parallel JoVE contribution by Axmann et al.	
RPMI 1640, with L-Glutamine	Life Technologies	11554416	T-cell media
non-essential amino acid 100X	Hyclone	SH30238.01	T-cell media supplement
penicillin/streptomycin/L-glutamine 100x	Life Technologies	12000226	T-cell media supplement
2-mercaptoethanol	Sigma Aldrich	M6250	T-cell media supplement
mouse interleukin-2 recombinant protein	BPS Bioscience	90185-B	T-cell media supplement
Research Grade Fetal Bovine Serum	Hyclone	SV30160.03	T-cell media supplement
Origin (analysis program)	OrigenLab	http://www.originlab.com/	non-linear fitting of two parameters (tauoff, [ntlag])