

Materials List for:

# Optimized Staining and Proliferation Modeling Methods for Cell Division Monitoring using Cell Tracking Dyes

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URL: <https://www.jove.com/video/4287>

DOI: [doi:10.3791/4287](https://doi.org/10.3791/4287)

## Materials

Name	Company	Catalog Number	Comments
<b>Commercially Purchased</b>			
7-Aminoactinomycin D	 Sigma-Aldrich	A9400	
Bovine serum albumin (BSA)	 Sigma-Aldrich	A4503	
Fetal bovine serum (FBS)	Atlanta Biologicals	S11150	
Hanks balanced salt solution (HBSS)	Life Technologies	14175-079	Calcium and magnesium free, without phenol red
Human IgG Cohn fraction II and III globulins	 Sigma-Aldrich	G-4386	
Mouse anti-human CD3-FITC	BD Biosciences	349201	Saturating concentration as determined by laboratory titration
Mouse anti-human CD4-APC	BD Biosciences	340672	Saturating concentration as determined by laboratory titration
Mouse anti-human CD4-PECy7	BD Biosciences	348799	Saturating concentration as determined by laboratory titration
Mouse anti-human CD8-FITC	BD Biosciences	347313	Saturating concentration as determined by laboratory titration
Mouse anti-human CD8-APC	Caltag (Life Technologies)	MHCD0805	Saturating concentration as determined by laboratory titration
Mouse anti-human CD19-APC	Caltag (Life Technologies)	MHCD1905	Saturating concentration as determined by laboratory titration
Mouse anti-human CD25-APC	BD Biosciences	340938	Saturating concentration as determined by laboratory titration
Mouse anti-human CD127-PE	BD Biosciences	557938	Saturating concentration as determined by laboratory titration
Mouse anti-human CD3	eBiosciences	16-0037-85	1.0 mg/mL; azide free
Mouse anti-human CD28	eBiosciences	16-0289-85	1.0 mg/mL; azide free
PBS	Gibco	21300-058	
PKH26 red fluorescent cell linker kit containing 10-3M PKH26 in EtOH and Diluent C	 Sigma-Aldrich	PKH26GL-1KT or MINI26-1KT	Procedures in Step 1 also apply to kits containing PKH67 or other CellVue dyes
CellVue Claret far red fluorescent cell linker kit containing 10-3M CellVue Claret in EtOH and Diluent C	 Sigma-Aldrich	MINCLARET-1KT or MIDCLARET-1KT	
5-(and-6)-carboxyfluorescein diacetate, succinimidyl ester (CFDA-SE)	Invitrogen (Life Technologies)	C34554	Non-fluorescent; cleaved by membrane esterases to form fluorescent amino-reactive

			carboxyfluorescein succinimidyl ester (CFSE)
LIVE/DEAD Fixable Violet	Invitrogen (Life Technologies)	L34955	
Sterile 12x75 mm conical polypropylene tubes & caps	VWR	60818-102	Gives better membrane dye staining efficiency (reduced dye adsorption; less cell loss during supernatant aspiration)
12x75 mm round bottom polystyrene tubes	Becton Dickinson	21008-936	
Flow cytometer	BD Bioscience	FACSCalibur LSRFortessa	Any cytometer able to detect FITC, PKH26, and 7-AAD (ex. 488 nm; em. 520 nm, 567nm, and 655nm, respectively) and APC ex. 633-640 nm; em. 660nm)
Flow cytometer	Beckman Coulter	LSRII CyAn	Any cytometer able to detect FITC, PKH26, and 7-AAD (ex. 488 nm; em. 520 nm, 567nm, and 655nm, respectively) and APC ex. 633-640 nm; em. 660nm)
<b>Laboratory Prepared</b>			
7-Aminoactinomycin D, concentrated stock	NA	NA	1 mg/mL in PBS. Freeze in aliquots and store at -20 °C.
7-Aminoactinomycin D, working stock	NA	NA	100 µg/mL in PBS; prepare daily from 1 mg/mL frozen stock.
IgG block	NA	NA	HBSS + 10 mg/mL Human IgG Cohn fraction II and III globulins + 10 mg/mL BSA.