

In Vivo Immunogenicity Screening of Tumor-Derived Extracellular Vesicles by Flow Cytometry of Splenic T Cells

Florian Stritzke^{1,2}, Hendrik Poeck^{1,2,3,4}, Simon Heidegger^{1,2}

¹Department of Medicine III, School of Medicine, Technical University of Munich ²Center for Translational Cancer Research (TranslaTUM), School of Medicine, Technical University of Munich ³Department of Internal Medicine III, University Hospital Regensburg ⁴National Centre for Tumor Diseases WERA

Corresponding Authors

Florian Stritzke
f.stritzke@tum.de

Simon Heidegger
simon.heidegger@tum.de

Citation

Stritzke, F., Poeck, H., Heidegger, S. *In Vivo Immunogenicity Screening of Tumor-Derived Extracellular Vesicles by Flow Cytometry of Splenic T Cells. J. Vis. Exp.* (175), e62811, doi:10.3791/62811 (2021).

Date Published

September 23, 2021

DOI

10.3791/62811

URL

jove.com/video/62811

Materials

Name	Company	Catalog Number	Comments
Anti-CD3 FITC	Biolegend	100204	Clone 17A2
Anti-CD4 PacBlue	Biolegend	100428	Clone GK1.5
Anti-CD8 APC	Biolegend	100712	Clone 53-6.7
Anti-IFN γ PE	eBioscience	RM90022	Clone XMG1.2
Brefeldin A	Biolegend	420601	Brefeldin A Solution (1,000x)
Cell Strainer, 100 μ m	Greiner	542000	EASYstrainer 100 μ m
DMEM	Sigma-Aldrich	D6429	Dulbecco's Modified Eagle's Medium with D-glucose (4.5 g/L) and L-glutamine (4 mM)
FBS Good Forte	PAN BIOTECH	P40-47500	Fetal Calf Serum (FCS)
Fixable Viability Dye eFluor 506	eBioscience, division of Thermo Fischer Scientific	65-0866-14	
Fixation/Permeabilization Concentrate	eBioscience	00-5123-43	Fixation/Permeabilization Concentrate (10x)
Fixation/Permeabilization Diluent	eBioscience	00-5223-56	
Ionomycin	Sigma-Aldrich	407952	From Streptomyces conglobatus - CAS 56092-82-1, $\geq$ 97% (HPLC)
L-Glutamine	Gibco	25030-032	L-Glutamine (200 mM)
Ovalbumin	InvivoGen	vac-pova	Ovalbumine with < 1 EU/mg endotoxin - CAS 9006-59-1
Oxaliplatin	Pharmacy of MRI hospital		
PBS	Sigma-Aldrich	D8537	Phosphate Buffered Saline without calcium chloride and magnesium chloride
Penicillin-Streptomycin	Gibco	1514-122	Mixture of penicillin (10,000 U/mL) and streptomycin (10,000 ug/mL)
PMA	Sigma-Aldrich	P1585	Phorbol 12-myristate 13-acetate, $\geq$ 99% (HPLC)

PVDF filter, 0,22 µm, for syringes	Merck Millipore	SLGV033RS	Millex-GV Filter Unit 0.22 µm Durapore PVDF Membrane
Red Blood Cell Lysis Buffer	Invitrogen	00-4333-57	
RPMI 1640	Thermo Fischer Scientific	11875	Roswell Park Memorial Institute 1640 Medium with D-glucose (2.00 g/L) and L-glutamine (300 mg/L), without HEPES
Syringe, 26 G	BD Biosciences	305501	1 mL Sub-Q Syringes with needle (0.45 mm x 12.7 mm)
Total Exosome Isolation Reagent	Invitrogen	4478359	For isolation from cell culture media
β-Mercaptoethanol	Thermo Fischer Scientific	31350	β-Mercaptoethanol (50 mM)