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UT Southwestern - Staining Melanoma Cells for Flow Cytometry

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1 *Works for me* This protocol is published without a DOI.

NCI PDMC consortium

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PROTOCOL CITATION

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PARENT PROTOCOLS

In steps of

UT Southwestern - Human Melanoma Metastatic Potential in Mice

STAINING CELLS FOR FLOW CYTOMETRY

1 Adjust staining volume and antibody concentration to the number of cells. Concentrations listed are for $5x10^6$ cells in a 50ml reaction. Do not exceed 10^6 cells/10ml.

- mLin APC: Ter119 (1/100), CD31(1/100), CD45(1/200)
- HLA FITC: HLA-ABC (1/5)

2 Add antibodies to tube and incubate covered on ice for **00:20:00**

3 Wash 1x with **1 mL** staining medium and spin down **220 rpm, 4°C 00:04:00** . Aspirate supernatant

4 DAPI staining: resuspend samples in DAPI 1:2000 to gate out dead cells. Stock in made in water (1mg/ml)

FACS

5 Adjust final sample volume to run at 6000 events/sec at maximum and then run. If clogging becomes an issue, then filter through cut pieces of filter

Xenograft stain (50ml volume)	1x	5x
HLA- PE or FITC (1/5)	10ml	50ml
mCD45 APC (1/200)	0.3ml	1.5ml
mCD31 APC (1/100)	0.5ml	2.5ml
mTer119 APC (1/100)	0.5ml	2.5ml

Human stain (50ml volume)	**1x**	**5x**
HLA- FITC (1/5)	10ml	50ml
ISO PE or P75 PE (1/5)	10ml	50ml
Glyc-A APC (1/2000)	0.025ml	0.125ml
hu-CD31 APC (1/800)	0.063ml	0.315ml
hu-CD45 APC (1/5)	10ml	50ml