CELLS FOR PRECISION MEDICINE.

Application Note

In-Cassette Cell Identification and Immunofluorescence Staining using the Parsortix ™ Platform

Introduction

The procedures described in this document are for RESEARCH USE ONLY and not for use in clinical or diagnostic procedures.

Immunofluorescence

Immunofluorescence Microscopy (IF) is a commonly used technique that can be performed on cells captured using the Parsortix™ platform and enables identification and phenotypic characterisation of CTCs.

Here we describe an IF workflow for the identification of CTCs based on accepted criteria of Cytokeratin positive (CK^+), CD45 negative ($CD45^-$) and a well-defined nucleus as determined by NucBlue staining. 2103558586

Reagents and equipment

Product	Catalog number	Supplier
Reagents		
DPBS	21-030-CV	Corning
Triton X-100	X-100	Sigma
16% Paraformaldehyde (PFA)	15710	Electron Microscopy Sciences
Bovine Serum Albumin (BSA)	A7030	Sigma
Pan-Keratin (clone C11) Mouse mAb Alexa488	4523S	Cell Signalling
PE/Dazzle anti-human CD45 (Clone HI60)	304052	BioLegand
NucBlue Live Cell Stain [Hoechst33342]	37605	Invitrogen
Fluorescence Cubes		
Fluorescence cube (Green)	39002 (or equivalent)	Chroma
Fluorescence cube (Red)	39010 (or equivalent)	Chroma
Fluorescence cube (UV/Blue)	39000 (or equivalent)	Chroma

Buffers:

Staining Buffer: PBS with 0.1% Triton and 3% BSA. Store at 4°C when not in use.

4% PFA: 1 to 2 of 16% PFA in PBS; make fresh on a weekly basis

Methods

Parsortix Separation

1. Perform cell separation on Parsortix instrument, following the instructions in the User Manual and Quick Reference Guide

Staining Process

1. Prepare the following tubes for staining assay:

Parsortix Reagent Line	Reagent Name	Volume	Reagent Composition
1	4% Fixative (Paraformaldyhyde)	1mL	1 to 2 of 16% PFA in PBS; make fresh on a weekly basis
2	Permerabilization/Block	2mL	PBS with 0.1% Triton and 3% BSA. Store at 4°C when not in use.
3	Antibody Solution ‡	1mL	0.98mL of Staining buffer 12.5µL of Pan-Keratin antibody ‡ 5µL of CD45 antibody ‡ 1 drop of NucBlue

‡: Protect from Light

2. Place the capped reagents tubes onto the instrument the line labels and feeding each line through the appropriate tube cap. Note that the antibody solution must be protected from light and this can be accomplished by wrapping the tube in aluminium foil.
3. Place a piece of aluminium foil over the clamp.
4. Run the instrument protocol PX2_cassetteIF.seq
5. The staining assay will run and prompt the user when staining is complete.
6. Visualize with fluorescence microscope. CK^+ cells visually appear green; with emission and excitation peaks at 499 nm and 520 nm, respectively. $CD45^+$ cells visually appear red; with emission and excitation peaks at 566 nm and 610 nm.

Sample outcomes

Figure 1: SKBR3 cultured cells separated and stained inside the Parsortix cassette *Representative images of results. Taken using Leica DMI5000 fluorescence microscope with 20X objective coupled.*

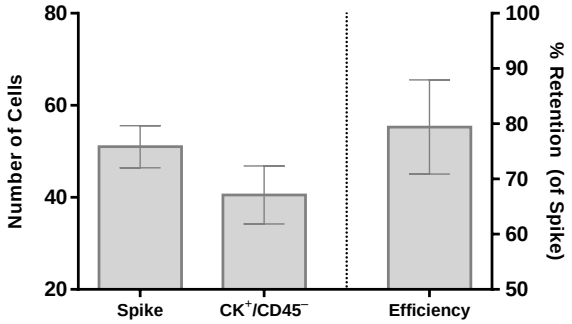

Figure 2: Efficiency of in-cassette staining assay. *This data shows the number of spiked and identified Sk-Br-3 cells processed via 2 operators. The right y-axis shows the calculated % cell retention (n=17).*

Troubleshooting

Assay Troubleshooting

Concern: There are no cells visible at the end of staining assay

When working with rare cells there is always a risk of cell loss. It is extremely important to be careful throughout the process to ensure best results. When a new lab or user begins work with this assay, ANGLE recommends running a control sample with 500 spiked cells alongside experimental conditions and controls to help troubleshoot the source of any potential loss. These cells can be added directly to a separate chamber slide, then fixed/stained alongside any other samples.

Instrument Troubleshooting

See Parsortix User Manual for separation or general instrument troubleshooting.

Contact ANGLE technical support at us-support@angleplc.com or eu-support@angleplc.com for additional troubleshooting.