Blood sample preparation
- Collect 6-9 mL of whole blood.
- Dilute samples to 20mL with PBS-fibrin.

Circulating rare cell isolation
- Filter diluted blood through the membrane at 10-30 mbar.
- Wash cells with PBS.
- Fix cells with formaldehyde (20 min).

ICC stain 1
- Permeabilize cells with 0.2% Triton-X solution in PBS (7 min).
- Incubate in blocking buffer (25 min).
- Incubate with antibody conjugate solution (25 min) to stain for CK and CD45.
- Apply DAPI to stain nuclei.

Microscopic cell analysis
- Apply cover medium and cover slip.
- Examine fluorescence signals in the blue, green, red, and far-red channels.
- Determine thresholds using control spiked cancer cells and white blood cells.
- Detect and enumerate circulating tumor cells.

ICC stain 2
- Remove cover slip and repeat ICC steps to stain for VIM and CD144.
- Reapply cover slip and repeat microscopic analysis of stained cells.
- Detect and enumerate circulating mesenchymal cells and circulating endothelial cells.

ICC stain 3
- Remove cover slip and proceed with TSA steps to stain for PIWIL2 or TPBG/ST4.
- Incubate in hydrogen peroxide to remove peroxidases and white blood cells (30 min).
- Incubate with the antibody conjugate (25 min) then tyramide-Alexa 488 (25 min).
- Reapply cover slip and repeat microscopic analysis of stained cells.
- Detect and enumerate putative circulating stem cells.