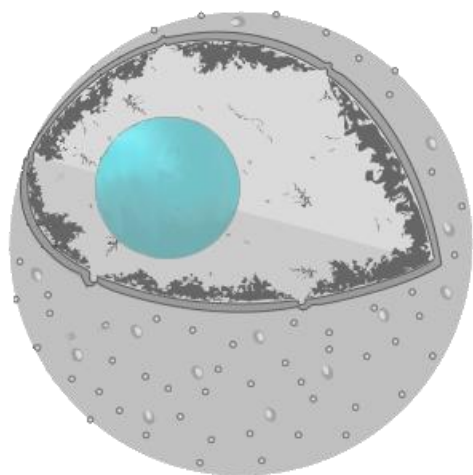


# Intranuclear Staining of Cells for ChipCytometry Quick Guide

Below are guidelines for the permeabilization of cells or tissues fixed on ZellSafe™ chips for staining of intranuclear markers with the True Nuclear™ Transcription Factor Buffer Set (CAT 424401) from BioLegend.



This protocol is for the intranuclear staining of cellular markers. For intracellular staining only, see our Intracellular Staining of Cellular Markers protocol.

## A. Preparing Solutions and Chip

1. Prepare a 1x working solution of the Perm Buffer by diluting the 10x Perm Buffer with **ZELLKRAFTWERK wash buffer**.
2. Freshly prepare 1x Fix working solution by diluting the 4x Fix Concentrate (1 part) with the Fix Diluent (3 parts). 1000  $\mu$ L are needed per chip.
3. Rinse chip that contains fixed cells/tissues with at least 200  $\mu$ L of **ZELLKRAFTWERK wash buffer** to remove air bubbles and ensure channel flow through.

## B. Permeabilizing the Cells

1. Rinse chip with 1000  $\mu$ L of 1x Fix Buffer.
2. Incubate at room temp for 20 min.
3. Wash chip with 1000  $\mu$ L of 1x Perm Buffer.
4. Dilute antibodies as titrated in appropriate volume of 1x Perm buffer and mix. For ZellSafe™ Cell chips, use a total volume of 300  $\mu$ L. For ZellSafe™ Rare or Tissue chips, use a total volume of 600  $\mu$ L for staining.
5. Incubate at room temp for 15 min.
6. Wash with 1000  $\mu$ L of 1x Perm buffer.
7. Wash at least 5 min with ZELLKRAFTWERK wash buffer (~5 mL). Wait 2 min and wash again for 5 min with ZELLKRAFTWERK wash buffer (~5 mL) right before scanning.
8. Scan on ZellScanner ONE. Repeat washing if back diffusion of antibody is observed during the scan.