This is a suggested procedure, please adjust according to your experimental needs.

Protocol aim

The aim of this protocol is to provide instructions to perform a staining using the KI67 and Vimentin antibodies accurately and reproducibly. Cells will be suspended in their appropriate medium.

Material:

Primary antibodies:

KI67 polyclonal antibody: Life Technologies Catalog #PA519462

Vimentin monoclonal antibody (J144): Invitrogen Catalog # MA3-745

Secondary antibodies:

Goat anti-rabbit IgG, DyLight™ 594: ThermoScientific #UJ291725

Goat anti-mouse IgG (H&L), DyLight™ 488: ThermoScientific #UJ291725

Dyes:

Hoechst 33343: ThermoScientific, #62248

Reagents:

Power Block, BioGenex, #HK0855K

Day 1

Seeding of cells according to protocol:

Dispensing Cells with I-DOT One

Dispensing Cells with I-DOT Mini

Day 2

Staining to be performed 20 h or more (up to 72 h tested) after seeding the cells on DMA

1) Remove medium and wash the slide three times by immersing the DMA with ice cold PBS in an appropriate staining jar. Choose volume according to the size of the jar for this and the following steps.
2) Fix slide with 4% Formalin in dd H_2O at room temperature in a staining jar.
3) Wash DMA 3x with TBS in a staining jar.
4) Incubate DMA in 0,1% Triton X-100 in TBS (9,9 ml TBS + 100 µl Triton X-100) for 15 min in a staining jar.
5) Wash DMA 3x with TBS-Tween (0,1% Tween 20 in TBS) in a staining jar.
6) Incubate DMA 5 min with Power Block (1:10 diluted in dd H_2O) directly on the DMA by adding 500µL and carefully distribute across the entire DMA using a parafilm.
7) Remove remining liquid with vacuum

8) Incubate 1 h with the primary antibody at RT directly on the DMA by adding 300µL and carefully distribute across the entire DMA using a parafilm.
   Ki67: 1:100 diluted in PBS
   Vimentin 1:100 diluted in PBS
9) Wash 3x with TBS-Tween in a staining jar.
10) Add the secondary antibody for 30 min directly on the DMA by adding 500 µL and carefully distribute across the entire DMA using a parafilm.
   KI-67 - goat anti-rabbit 594 diluted 1:500 in PBS
   Vimentin - Goat anti mouse 488 diluted 1:500 in PBS
   Add Hoechst 33342 diluted 1:5000 in PBS
11) Wash 3x with TBS in a staining jar.
12) Embed with Mowiol

Preparation of Mowiol:

Reagent	Amount for 50 mL	Final conc.
Glycerol, puriss p.a.	12 g	24% (w/v)
H_2O	12 mL	--
Mowiol 4-88	4.8 g	9.6% (w/v)
Tris-Cl (0.2 M, pH 8.5)	24 mL	0.1 M

1. Mix glycerol and Mowiol; dissolve with frequent agitation for 1 h at room temperature.
2. Add H_2O. Stir for 1h at room temperature.
3. Add Tris-Cl. Incubate for 2 h at 50°C with occasional stirring, e.g., for 2 min every 15 min.
4. Centrifuge at 5000*g* for 15 min.
5. Store aliquots of the supernatant for up to 12 months at -20°C.

Note: Mowiol may not dissolve completely.