

**GENERAL GUIDELINES:**

- Reactions volume depends on the sample size.
- Use best practices when pipetting to minimize reagents consumption.
- Centrifuge vials before pipetting.
- The final working concentration of different detergents for permeabilization (ex. Tween-20, Triton X100, digitonin) should be explored to find the optimal conditions to best preserve the cell structure and protein of interest.
- Select the Buffer C vial with the appropriate detection fluorophore for your microscope filter set.
- Available fluorophores are Texas Red, Atto 647, and Atto 488. The standard kit is equipped with Texas Red; if other fluorophore is needed, contact Navinci Diagnostics at contact@navinci.se
- Thoroughly defrost all buffer mixtures at room temperature, vortex well before use.
- Vortex and spin down all enzymes (A, B, and C) before use.
- Keep enzymes on ice or a frozen cold block.
- Wait to add enzymes until immediately before adding to the sample.
- Buffer C is a light-sensitive reagent. Always keep it protected from light.
- Remove excess washing buffer from samples before adding reagent.
- Do not allow slides/samples to dry.
- Preheat the humidity chamber before each step.
- Incubation times or temperatures other than those specified may give erroneous results.
- Naveni assays might be combined with traditional immunofluorescence techniques, providing that all primary antibodies are from different species and the fluorescence detection systems use different fluorophores.
- As with any product derived from biological sources, proper handling procedures should be used.
- Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.
- Unused solutions should be disposed of according to local regulations.

IMPORTANT: Cross-contamination is the main source of unspecific background due to the high sensitivity of the assay.

KIT COMPONENTS:**Box 1:**

Material	Article Number	Amount	Storage*
Blocking Buffer (1x)	NF.1.100.01	4000 µl	at +4 to +8°C DO NOT FREEZE!!!
Navenibody Diluent (1x)	NPT.1.100.01	4000 µl	

Box 2:

Material	Article Number	Amount	Storage*
VEGFR2 Navenibody (40x)	NPT.2.22	100 µl	at -25 to -15°C
pTyr R Navenibody (40x)	NPT.2.21	100 µl	
Buffer A (5x)	NF.2.100.08	800 µl	
Enzyme A (40x)	NF.2.100.09	100 µl	
Buffer B (5x)	NF.2.100.10	800 µl	
Enzyme B (40x)	NF.2.100.11	100 µl	
Buffer C (5x), Texas Red	NF.2.100.12	800 µl	
Enzyme C (40x)	NF.2.100.15	100 µl	

* When stored as directed, the product is stable at least for three months after receipt



1. Permeabilization (not provided)
 - 1.1 Only for fresh frozen cell slides: Permeabilize cells with 0,05% Triton X-100 in PBS for 5 min at room temperature.
 - 1.2 Wash slides for 2x2 min with 1x PBS.
2. Blocking
 - 2.1 Add **Blocking Buffer** (1x) to the entire sample area (approximately 40 μ l for each 1cm² area).
 - 2.2 Incubate for 30 min at +37 °C in a pre-heated humidity chamber.
3. Navenibody incubation
 - 3.1 Prepare Navenibodies by diluting **VEGFR2 Navenibody (40x)** and **pTyr R Navenibody (40x)** in **Navenibody Diluent** (1x) (dilute 1:40 each).
 - 3.2 Add enough of the Navenibodies to cover the sample area.
 - 3.3 Incubate for 60 min at +37 °C in a pre-heated humidity chamber.
 - 3.4 Decant the solution and wash slides for 3x5 min with 1x TBS-T in a staining jar under gentle agitation.
4. Reaction A
 - 4.1 Start preparing **Reaction A** by diluting **Buffer A** (5x) 1:5 in water. Vortex and spin down.
 - 4.2 Add **Enzyme A** (dilute 1:40). Mix gently by pipetting and spin down.
 - 4.3 Add enough Reaction A to cover the sample area.
 - 4.4 Incubate for 60 min at +37 °C in a pre-heated humidity chamber.
 - 4.5 Decant the solution and wash slides for 2x3 min with 1x TBS-T in a staining jar under gentle agitation.
5. Reaction B
 - 5.1 Start preparing **Reaction B** by diluting **Buffer B** (5x) 1:5 in water. Vortex and spin down.
 - 5.2 Add **Enzyme B** (dilute 1:40). Mix gently by pipetting and spin down.
 - 5.3 Add enough Reaction B to cover the sample area.
 - 5.4 Incubate for 30 min at 37 °C in a pre-heated humidity chamber.
 - 5.5 Wash slides for 2x3 min with 1x TBS-T in a staining jar under gentle agitation.
6. Reaction C
 - 6.1 Select the **Buffer C** vial with the appropriate detection fluorophore for your microscope filter set. Do not use more than one **Buffer C** vial.
 - 6.2 Start preparing **Reaction C** by diluting **Buffer C** (5x) 1:5 in water. Vortex and spin down.
 - 6.3 Add **Enzyme C** (dilute 1:40). Mix gently by pipetting and spin down.
 - 6.4 Add enough Reaction C to cover the sample area.
 - 6.5 Incubate for 90 min at +37 °C in a pre-heated humidity chamber.
 - 6.6 Decant the solution and wash slides for 2 min with 1x TBS in a staining jar under gentle agitation.

Protect from light
7. Nuclei staining (not provided)
 - 7.1 Start preparing a Nuclei staining solution according to the manufacturer's instruction. Vortex and spin down.
 - 7.2 Decant wash buffer from the slides.
 - 7.3 Add enough Nuclei staining solution to cover the sample area.
 - 7.4 Incubate according to the manufacturer's instruction.
 - 7.5 Decant the solution and wash slides for 2x 10 min with 1x TBS in a staining jar under gentle agitation.
 - 7.6 Wash slides for 15 min with 0.1x TBS in a staining jar under gentle agitation.

Protect from light
8. Mounting (not provided)
 - 8.1 Decant excess wash buffer from the slides.
 - 8.2 Mount the slides with a coverslip using a Fluoroshield anti-fade mounting medium.
 - 8.3 Image your slides in fluorescence or confocal microscope, using 20x objective or higher and filter set for DAPI and a corresponding fluorophore (FITC for Atto 488, Texas Red, or Cy5 for Atto 647, respectively).

Protect from light

**TBS-T (Tris-buffered saline supplemented with 0.05% Tween 20)