



## 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](mailto: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<br>+4 to +8°C<br>DO NOT<br>FREEZE!!! |
| Navenibody Diluent (1x) | NPT.1.100.01   | 4000 µl |   |

### Box 2:

| Material                 | Article Number | Amount | Storage*           |
|--------------------------|----------------|--------|--------------------|
| PDGFRβ Navenibody (40x)  | NPT.2.20       | 100 µl | at<br>-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 µl for each 1cm<sup>2</sup> 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 **PDGFRβ 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)