

NPT.EGFR

# Naveni pTyr EGFR

## 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.

## Kit components

### Box 1:

Storage: +4 to +8°C.



| Material                | Art.no       | Amount  |
|-------------------------|--------------|---------|
| Blocking Buffer (1x)    | NF.1.100.01  | 4000 µl |
| Navenibody Diluent (1x) | NPT.1.100.01 | 4000 µl |

### Box 2:

Storage: -25 to -15°C. Protect from light.



| Material                 | Art.no      | Amount |
|--------------------------|-------------|--------|
| EGFR Navenibody (40x)    | NPT.2.17    | 100 µl |
| 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 3 months after receipt.



### Important:

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



For more information, or to place an order, visit [www.navinci.se/products](http://www.navinci.se/products)  
Email: [contact@navinci.se](mailto:contact@navinci.se)

# Instructions for use

## 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 **EGFR 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.

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

## 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: Protect from light!

- 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.

## 7. Nuclei staining (not provided): Protect from light!

- 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.

## 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).