SZABO SCANDIC

Part of Europa Biosite

# Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

## Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

## Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand


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## 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.

# Naveni pTyr EGFR KIT INSTRUCTIONS

Item No. 39206

**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* |
|---|---|---|---|
| **EGFR Navenibody (40x)** | NPT.2.17 | 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 | |

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|---|---|---|
| 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$^2$ 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. |
| 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. |
| **Protect from light** | 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) | 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. |
| **Protect from light** | 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. |
| **Protect from light** | 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). |

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