Item Numbers 39220 & 39221

# NaveniFlex Tissue GR

Navinci

## General guidelines

- Reaction volume depends on sample size and should be sufficient to cover the entire sample area. A volume of approximately 40 µl/cm ² is recommended. For example, for an FFPE tissue section that covers an area of 5 cm ² on a slide, we recommend you use a reaction volume of 200 µl/slide.
- Use best practices when pipetting to minimize reagent consumption.
- Thoroughly defrost all buffers at room temperature. Vortex well before use.
- Centrifuge vials before pipetting.
- Spin down Enzymes 1 and 2 before use.
- Keep enzymes on ice or on a frozen cooling block.
- Add enzymes right before adding reaction mix to sample.
- The recommended Navenibody NT working dilution is 1:80. Depending on target abundance, Navenibodies can be titrated up to 1:40 or down to 1:160.
- The NaveniFlex Tissue GR kit can be combined with traditional immunofluorescence (IF), provided all primary antibodies are either raised in different species, or labelled with fluorophores different from the ones used in the kit.
- Select the Detection reagent NT with the appropriate detection fluorophore for your microscope filter set. Consider fluorophore compatibility and bleed-through if performing an immunofluorescence co-stain.
- Detection reagent NT is light-sensitive. Always keep it protected from light.
- Remove excess washing buffer from samples before adding the reagent mix.
- Do not allow slides/samples to dry.
- Preheat humidity chamber before each step.
- Incubation times or assay temperatures other than those specified may negatively impact results.
- As with any product derived from biological sources, proper handling procedures should be used.
- Wear appropriate personal protective equipment to avoid contact of reagents with eyes and skin.
- Unused solutions should be disposed of according to local regulations.

### Important:

Appropriate precautions should be taken to avoid antibody cross-contamination. Cross-contamination is the primary source of unspecific background due to the high sensitivity of the assay.

Avoid bulk washing methods when different primary antibody pairs are used, or when washing different technical controls.

## Kit components

### Box 1:

Storage: 4 to 8°C

DO NOT FREEZE

| Material | Art.no | Amount |
|---|---|---|
| Block NT (1X) | NT.1.100.01 | 4 ml |
| Diluent 1 NT (1X) | NB.1.100.02 | 8 ml |
| Diluent 2 NT (1X) | NF.1.100.03 | 4 ml |
| Navenibody G1 NT (80X) | NB.1.100.18 | 100 µl |
| Navenibody R2 NT (80X) | NB.1.100.07 | 100 µl |
| Post-block NT (1X) | NF.1.100.01 | 4 ml |

### Box 2:

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

FREEZE

| Material | Art.no | Amount |
|---|---|---|
| Buffer 1 NT (5X) | NB.2.100.17 | 800 µl |
| Enzyme 1 NT (40X) | NF.2.100.11 | 100 µl |
| Buffer 2 NT (5X) | NT.2.100.01 | 800 µl |
| Enzyme 2 NT (40X) | NF.2.100.15 | 100 µl |
| Post-block supplement NT (40X) | NT.2.100.04 | 100 µl |
| Detection reagent NT** (5X), Red/ Atto647N | NT.2.100.02/ NT.2.100.03 | 800 µl |

*When stored as directed, the product is stable for at least 3 months after receipt.

**Depending on the kit you purchased, either the Red or the Atto647N Detection Reagent NT will be included.

Distributed by:
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# Instructions of use

## 1. Pre-treatment (reagents not provided)

1.1 Perform the appropriate pre-treatment steps for your sample type (e.g., deparaffinization and antigen retrieval for FFPE tissues) according to your standard immunohistochemistry protocol.
1.2 Where applicable, use a hydrophobic barrier pen around sample to prevent spillage and sample drying.

## 2. Blocking

2.1 Add Block NT to the entire sample area.
2.2 Incubate for 60 min at 37 °C in a preheated humidity chamber.

## 3. Primary antibody incubation

3.1 Use the provided Diluent 1 NT to dilute your primary antibodies to working concentration. Start with a concentration optimized for/ recommended by the antibody vendor for IHC.
3.2 Decant the Block NT and add a sufficient volume of the antibody working solution from step 3.1 to cover the sample area.
3.3 Incubate for 60 min at 37 °C or overnight at 4 °C in a humidity chamber.
3.4 Decant the antibody solution, wash slides 2x 10 sec and 1x 15 min with 1X TBS-T [1] in a staining jar under gentle agitation. Wash controls separately.

## 4. Navenibody incubation

4.1 Dilute Navenibody G1 NT and Navenibody R2 NT 1:80 in Diluent 2 NT.
4.2 Add a sufficient volume of the Navenibody working solution from step 4.1 to cover the sample area.
4.3 Incubate for 60 min at 37 °C in a preheated humidity chamber.
4.4 Decant the solution and wash slides 2x 10 sec and 1x 15 min with 1X TBS-T in a staining jar under gentle agitation. Wash controls separately.

## 5. Reaction 1 (for an example calculation for a total reaction volume of 200 µl, sufficient for a sample area of ~5 cm ², see table in 5.2.)

5.1 Start preparing Reaction 1 by diluting Buffer 1 NT 1:5 in water. Vortex and spin down.
5.2 Add Enzyme 1 NT so that it is diluted 1:40. Mix gently by pipetting and spin down.

| Reagent for 5 cm² sample area | Volume |
|---|---|
| $H_2O$ | 155 µl |
| Buffer 1 NT (5x) | 40 µl |
| Enzyme 1 NT (40x) | 5 µl |
| Total volume: | 200 µl |

5.3 Add a sufficient volume of Reaction 1 to cover the sample area.
5.4 Incubate for 30 min at 37 °C in a preheated humidity chamber.
5.5 Decant the solution, wash slides 1x 10 sec and 1x 5 min with 1X TBS-T in a staining jar under gentle agitation.

## 6. Reaction 2: protect from light!

6.1 Start preparing Reaction 2 by diluting Buffer 2 NT 1:5 in water. Vortex and spin down.
6.2 Add Enzyme 2 NT so that it is diluted 1:40. Mix gently by pipetting and spin down.
6.3 Add a sufficient volume of Reaction 2 to cover the sample area.
6.4 Incubate for 90 min at 37 °C in a preheated humidity chamber. Proceed to step 7.1 before the incubation is over.

## 7. Post-block [2]

7.1 While Reaction 2 is incubating, thaw and dilute Post-block supplement NT 1:40 in Post-block NT . Vortex and spin down.
7.2 After the incubation step is complete, decant Reaction 2 and add a sufficient volume of the Post-block working solution from step 7.1 to cover the sample area.
7.3 Incubate for 30 min at 37 °C in a preheated humidity chamber.

## 8. Detection (protect from light!)

8.1 Prepare the Detection working solution by diluting Detection reagent NT 1:5 in water. Vortex and spin down.
8.2 Decant the Post-block solution. Do not wash at this step, but make sure the Post-block solution has been removed. Add a sufficient volume of Detection working solution from step 8.1 to cover the sample area.
8.3 Incubate for 30 min at 37 °C in a preheated humidity chamber.
8.4 Decant the solution and wash slides for 2 min with 1X TBS in a staining jar under gentle agitation.

## 9. Nuclear stain and mounting (reagents not provided; protect from light!)

9.1 Decant excess washing buffer from the slides.
9.2 Add DAPI or a nuclear stain of your choice with a similar emission spectrum diluted in PBS to a concentration recommended by the vendor. Incubate for 5 min at room temperature in a humidity chamber.
9.3 Decant the solution, wash slides 2x 10 min in 1X TBS under gentle agitation.
9.4 Perform a final 15 min wash in 0.1X TBS under gentle agitation. Dry slides in a slide centrifuge or air-dry them, and mount them with a coverslip using an antifade mounting medium.

## 10. Imaging

10.1 Image your slides on a fluorescence or confocal microscope using a 20x objective or higher.
10.2 For imaging, a filter set corresponding to your chosen nuclear stain and detection fluorophore's excitation/ emission spectra is required.

| Filter set | Detecting | λ Excitation | λ Emission |
|---|---|---|---|
| DAPI | Nuclei | | |
| FITC | Possible IF co-stain | 480-490 nm | 525-535 nm |
| Cy3 | Possible IF co-stain | 545-555 nm | 575-585 nm |
| TexasRed | Proximity signal detected by Detection Reagent NT Red | 585-595 nm | 615-625 nm |
| Cy5 | Proximity signal detected by Detection Reagent NT Atto647 | 635-645 nm | 665-675 nm |

[1] TBS-T (Tris-buffered saline supplemented with 0.05% Tween 20)

[2] If desired, it is possible to add fluorescently labelled antibodies for co-staining at this step.

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