NT.GR.100 (Red/Atto647N)

NaveniFlex™ Tissue GR

General guidelines

- Reaction volume depends on sample size and should be sufficient to cover the entire sample area. A volume of approximately 40 µL/cm² is recommended. For example, for an FFPE tissue section that covers an area of 5 cm² 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.
- Vortex and 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 working dilution is 1:40. Depending on target abundance, Navenibodies can be further titrated down to 1:80.
- Incubation times or assay temperatures other than those specified may negatively impact results. Warm washing buffer is only required for step 4.4, additional warm washing steps may lead to signal deterioration.
- The NaveniFlex Tissue GR kit can be combined with traditional immunofluorescence (IF), provided that 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 with the appropriate detection fluorophore for your microscope filter set. Consider fluorophore compatibility and bleed-through if performing an immunofluorescence co-stain.
- Detection reagent 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 (1X)	NT.1.100.01	4 mL
Diluent (1X)	NB.1.100.02	2X 8 mL
Navenibody G1 (40X)	NB.1.100.18	100 µL
Navenibody R2 (40X)	NB.1.100.07	100 µL

Box 2:

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

FREEZE

Material	Art.no	Amount
Buffer 1 (5X)	NB.2.100.17	800 µL
Enzyme 1 (40X)	NF.2.100.11	100 µL
Buffer 2 (5X)	NT.2.100.01	800 µL
Enzyme 2 (40X)	NF.2.100.15	100 µL
Post-block supplement (40X)	NT.2.100.04	100 µL
*Detection Reagent (5x), Red, or Detection Reagent (5x), Atto647N	NT.2.100.05/ NT.2.100.06	800 µL

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

For more information, or to place an order, visit
www.navinci.se/products
Email: contact@navinci.se

Instructions for 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.
1.3 Prepare two bottles of 1X TBS-T* and pre-warm one bottle to 37°C together with the required number of staining jars (warm wash is required after Navenibody incubation at Step 4.4).

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

2. Blocking

2.1 Add Block 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 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 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 in a staining jar under gentle agitation. Wash controls separately.

4. Navenibody incubation

4.1 Dilute Navenibody G1 and Navenibody R2 1:40 in Diluent.
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 the slides with the pre-warmed 1X TBS-T from Step 1.3. Wash for 2x 10 sec and 1x 15 min in the pre-warmed staining jar under gentle agitation. Wash the controls separately.

5. Reaction 1

For an example calculation for a total reaction volume of 200 µL, sufficient for a sample area of ~5 cm², see table 1.

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

Table 1. Example calculation for a 200 µL reaction volume.

Reagent for 5 cm² sample area	Volume
H_2O	155 µL
Buffer 1 (5x)	40 µL
Enzyme 1 (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 1:5 in water. Vortex and spin down.
6.2 Add Enzyme 2 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

7.1 While Reaction 2 is incubating, thaw and dilute Post-block supplement 1:40 in Diluent. 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[1] (protect from light!)

8.1 Prepare the Detection working solution by diluting Detection reagent 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 5 min in 1X TBS-T under gentle agitation.
9.4 Perform a final 5 min wash in 0.1X TBS-T 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, see table 2.

Table 2. Filter sets for imaging.

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 Red	585-595 nm	615-625 nm
Cy5	Proximity signal detected by Detection Reagent Atto647	635-645 nm	665-675 nm

[1](Optional) Fluorescently labelled primary antibodies may be added together with the detection working solution if co-staining is desired. Dilute and incubate as recommended by the manufacturer.

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