Art.no 60024

Naveni® TriFlex Cell

Instructions For Use

For research use only

Manufactured by:

Navinci Diagnostics AB
Uppsala Science Park
Dag Hammarskjölds väg 52A
SE-752 37 Uppsala
SWEDEN

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

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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 8-well chamber slide with a growth area of 0.93 cm²/well, we recommend you use a reaction volume of 40 µL/well.
- 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 Naveni Enzyme 1 (40x) and Naveni Enzyme 2 (40x) before use.
- Keep enzymes on ice or on a frozen cooling block.
- Add enzymes right before adding reaction mix to sample.
- Reaction 2 contains a light-sensitive reagent. 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:

Art. no. 60039
Storage: 2 to 8°C



Material	Art.no	Amount
Naveni Block	50014	6 mL
Naveni Diluent	50001	8 mL
Navenibody Diluent	50057	4 mL
TriFlex Navenibody M	50053	100 µL
TriFlex Navenibody R	50054	100 µL

Box 2:

Art. no. 60042
Storage: -25 to -15°C. Protect from light!



Material	Art.no	Amount
Naveni TriFlex Buffer 1 (5x)	50019	800 µL
Naveni Enzyme 1 (40x)	50002	100 µL
Naveni TriFlex Buffer 2 (5x)	50026	800 µL
Naveni Enzyme 2 (40x)	50003	100 µL

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1. Pre-treatment (reagents not provided)

1.1 Fix and permeabilize cells according to the immunofluorescence protocol optimized for the primary antibodies utilized in this assay.
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*; pre-warm one bottle to 37°C, preferably overnight, together with the required number of staining jars (a warm wash is required after Navenibody incubation at Step 4.4).

2. Blocking

2.1 Add Naveni 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 Naveni Diluent to dilute your primary antibodies to working concentration. Start with a concentration optimized for/ recommended by the antibody vendor for IF.
3.2 Decant the Naveni 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.

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

4. Navenibody incubation

4.1 Dilute TriFlex Navenibody M and TriFlex Navenibody R 1:40 in Naveni 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 Navenibody solution and wash slides with the prewarmed 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.

5. Reaction 1

For an example calculation for a total reaction volume of 40 µL, sufficient for a sample area of ~1 cm², see **table 1**.

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

Table 1. Example calculation for a 40 µL reaction volume.

Reagent for 1 cm² sample area	Volume
H_2O	31 µL
Naveni TriFlex Buffer 1 (5x)	8 µL
Naveni Enzyme 1 (40x)	1 µL
Total volume:	40 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 Naveni TriFlex Buffer 2 (5x) 1:5 in water. Vortex and spin down.
6.2 Add Naveni Enzyme 2 (40x) 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 60 min at 37 °C in a preheated humidity chamber.
6.5 Decant the solution and wash slides for 2 min with 1x TBS in a staining jar under gentle agitation.

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

7.1 Decant excess washing buffer from the slides.
7.2 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.
7.3 Decant the solution, wash slides 2x 10 min in 1x TBS under gentle agitation.
7.4 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.

8. Imaging

8.1 Image your slides on a fluorescence or confocal microscope using a 20x objective or higher.
8.2 For imaging, a filter set corresponding to DAPI, FITC, Cy3, and Cy5 is required, see **table 2**.

Table 2. Filter sets for imaging.

Filter set	Detecting	λ Excitation	λ Emission
DAPI	Nuclei		
FITC	Rabbit antibody signal	480-490 nm	525-535 nm
Cy3	Mouse antibody signal	545-555 nm	575-585 nm
Cy5	Proximity signal	635-645 nm	665-675 nm

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