TF.MR.100

Naveni® TriFlex Cell MR

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 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.
- 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:

Storage: 4 to 8°C

Material	Art.no	Amount
Block TF (1X)	NT.1.100.01	4 ml
Diluent 1 TF (1X)	NB.1.100.02	8 ml
Diluent 2 TF (1X)	NF.1.100.03	4 ml
Navenibody M TF (40X)	TF.1.100.04	100 µl
Navenibody R TF (40X)	TF.1.100.05	100 µl

Box 2:

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

Material	Art.no	Amount
Buffer 1 TF (5X)	TF.2.100.06	800 µl
Enzyme 1 TF (40X)	NF.2.100.11	100 µl
Buffer 2 TF (5X)	TF.2.100.07	800 µl
Enzyme 2 TF (40X)	NF.2.100.15	100 µl

When stored as directed, the product is stable for at least 6 months after receipt.

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

Uppsala August 31 2023

Instructions for use

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.

2. Blocking

2.1 Add Block TF 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 TF 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 Block TF 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 Navenibody M TF and Navenibody R TF 1:40 in Diluent 2 TF.
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.

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 in 5.2.)

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

Reagent for 1 cm² sample area	Volume
H_2O	31 µl
Buffer 1 TF (5x)	8 µl
Enzyme 1 TF (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 Buffer 2 TF 1:5 in water. Vortex and spin down.
6.2 Add Enzyme 2 TF 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.

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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