Navinci

TF.MR.100

# Naveni™ TriFlex Cell MR

## General guidelines

- Reaction volume depends on sample size.
- Use best practices when pipetting to minimize reagent consumption.
- Thoroughly defrost all buffer mixtures at room temperature, vortex well before use.
- Centrifuge vials before pipetting.
- Vortex and spin-down all enzymes (1 and 2) before use.
- Keep enzymes on ice or a frozen cold 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 reagent.
- Do not allow slides/samples to dry.
- Preheat the humidity chamber before each step.
- Incubation times or temperatures other than those specified may compromise results.
- 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:

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 multiple antibodies are used.

## 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.

| 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

# Instructions of use

## 1. Blocking

**1.1** Add Block TF (1x) to the entire sample area (approximately 40 µl/cm2).
**1.2** Incubate for 60 min at +37 °C in a preheated humidity chamber.

## 2. Primary antibody incubation

**2.1** Use the provided Diluent 1 TF (1x) to dilute your primary antibodies.
**2.2** Decant the Blocking Buffer and add enough of the antibody working solution from step 2.1 to cover the sample area.
**2.3** Incubate for 60 min at +37 °C or overnight at +4 °C in a humidity chamber.
**2.4** Decant the antibody solution, exchange wash twice, and wash slides for 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)

## 3. Navenibody incubation

**3.1** Dilute Navenibody M TF and Navenibody R TF 1:40 in Diluent 2 TF (1x).
**3.2** Add enough of the Navenibody working solution from step 3.1 to cover the sample area.
**3.3** Incubate for 60 min at +37 °C in a preheated humidity chamber.
**3.4** Decant the solution, exchange wash twice, and wash slides for 15 min with 1x TBS-T in a staining jar under gentle agitation.

## 4. Reaction 1

**4.1** Start preparing Reaction 1 by diluting Buffer 1 TF (5x) 1:5 in water. Vortex and spin down.
**4.2** Add Enzyme 1 TF (dilute 1:40). Mix gently by pipetting and spin down.
**4.3** Add enough Reaction 1 to cover the sample area.
**4.4** Incubate for 30 min at 37 °C in a preheated humidity chamber.
**4.5** Decant the solution, exchange wash once and wash slides for 5 min with 1x TBS-T in a staining jar under gentle agitation.

## 5. Reaction 2: protect from light!

**5.1** Start preparing Reaction 2 by diluting Buffer 2 TF (5x) 1:5 in water. Vortex and spin down.
**5.2** Add Enzyme 2 TF (dilute 1:40). Mix gently by pipetting, and spin down.
**5.3** Add enough Reaction 2 to cover the sample area.
**5.4** Incubate for 60 min at +37 °C in a preheated humidity chamber.
**5.5** Decant the solution and wash slides for 2 min with 1x TBS in a staining jar under gentle agitation.

## 6. Mounting (not provided): protect from light!

**6.1** Decant excess wash buffer from the slides.
**6.2** Add DAPI or a nuclear stain of your choice with a similar emission spectrum mixed in PBS. Incubate for 5 min at room temperature in a humidity chamber.
**6.3** Decant the solution, wash slides 2x10 min in 1x TBS under gentle agitation.
**6.4** Perform a final 15 min wash in 0.1x TBS under gentle agitation. Dry slides in a slide centrifuge and mount them with a coverslip using an anti-fade mounting medium.

## 7. Imaging

**7.1** Image your slides in fluorescence or confocal microscope, using 20x objective or higher.
**7.2** For imaging, a filter set corresponding to DAPI, FITC, Cy3, and Cy5 is needed.

| Filter set | Detecting | Excitation (λ) | Emmision (λ) |
|---|---|---|---|
| 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 |