

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.

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