NF.CK.MR

Naveni Control Kit

To be used with NaveniFlex Cell MR, NaveniFlex Tissue MR and NaveniBright

General guidelines

- Use a PAP pen to draw a sufficiently large border around each cell pellet.
- Reaction volume for control slides is 40 µl per cell pellet.
- Use best practices when pipetting to minimize reagents consumption.
- Centrifuge vials before pipetting.
- Completely defrost all buffer mixtures at room temperature, vortex well before use.
- Vortex and spin-down all enzymes (Enzyme 1 and Enzyme 2) before use.
- Keep enzymes on ice or on a frozen cold block.
- Wait to add enzymes until immediately prior to adding to the sample.
- 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 give erroneous 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 solution should be disposed according to local regulation.

Kit components

Box 1:

Storage: -25 to -15°C.

FREEZE

Material	Art.no	Supplier (Cat. No.)	Amount
Mouse anti-Her2 antibody (100X)	NF.2.CK.01	Origene (TA503443)	5 µL
Rabbit anti-Her2 antibody (100X)	NF.2.CK.02	Atlas Antibodies (HPA001383)	5 µL
Control Slides (BT474)	NF.2.CK.03	Acepix (N/A)	3 slides

Important:

Appropriate precautions should be taken to avoid antibodies cross-contamination. Cross-contamination is the main source of unspecific background due to the high sensitivity of the assay.

Avoid bulk washing methods when multiple antibodies are used.

Instructions for use

1. Slide preparation

1.1. Let slides warm up to room temperature.
1.2. Use a PAP pen and draw a border around each cell pellet and let the border dry completely.
1.3. Rehydrate the cells by adding 1x PBS onto each pellet, incubate for 3 min at room temperature.
1.4. Transfer slides into a staining jar with 1x PBS and wash for 2 min with gentle agitation.

2. Blocking

2.1. Add the Block (1x) from the Naveni kit of choice to the entire sample area (approximately 40 µL for each 1cm^2 area). If using NaveniBright, prepare blocking buffer with supplement 1.
2.2. Incubate for 60 min at 37 °C in a pre-heated humidity chamber.

3. Primary antibody incubation

3.1. Use the provided Diluent 1 (1x) to dilute the Mouse and Rabbit Primary Antibodies according to the table below. If using NaveniBright, prepare antibody diluent with supplement 2 and dilute the provided antibodies according to the table below. Note that when using the NaveniBright protocol there is an extra washing step between the blocking step (2) and primary antibody incubation step (3).

Antibody/ Diluent	Amount
Mouse anti-Her2 antibody (100x)	1 µL
Rabbit anti-Her2 antibody (100x)	1 µL
Diluent 1 (1x)	98 µL
Total:	100 µL

3.2. Decant the Block and add enough of the antibodies to cover one cell pellet – add 40 µL of Diluent 1 (1x) to the second cell pellet as negative control. See Figure 1 for a recommended slide layout.
3.3. Incubate overnight at +4 °C in a humidity chamber.
3.4. Aspirate to remove the antibody solution and wash slides for 3x5 min with 1x TBS-T* in a staining jar under gentle agitation.

4. Navenibody incubation

4.1. Continue with the Navenibody incubation step according to your specific Naveni kit instruction.

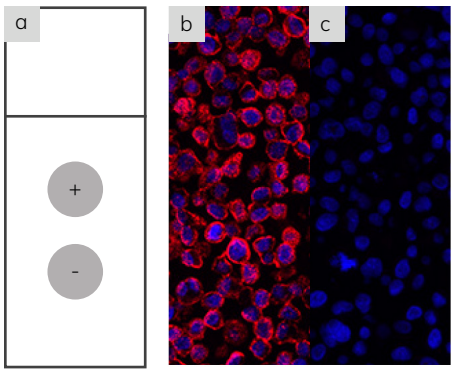


Figure 1. Recommended imaging settings
The recommended slide layout, where + indicates both primary antibodies present whilst - indicates no primary antibodies present **a).** Reference images displaying a typical positive **b),** and negative result **c).**

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

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