Navinci Control Kit KIT INSTRUCTIONS

To be used with NaveniFlex – MR Kit | Item No. 39205

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.
- Select the Buffer C vial with the appropriate detection fluorophore for your microscope filter set.
- Completely defrost all buffer mixtures at room temperature, vortex well before use.
- Vortex and spin-down all enzymes (A, B and C) before use.
- Keep enzymes on ice or on a frozen cold block.
- Wait to add enzymes until immediately prior to adding to the sample.
- Buffer C is 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 give erroneous results.
- NaveniFlex assay might be combined with traditional immunofluorescence techniques, providing that all primary antibodies are from different species and the fluorescence detection systems use different fluor ophores.
- 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.

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.

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CONTROL KIT COMPONENTS:

Material	Cat. No.	Supplier (Cat. No.)	Amount	Storage*
Mouse anti-Her2 antibody (100X)	NF.2.CK.01	Origene (TA503443)	5 µl	From -25 to -15°C
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	

PROTOCOL:

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 **Blocking Buffer** (1x) to the entire sample area (approximately 40 µl for each 1cm^2 area).

2.2 Incubate for 60 min at +37 °C in a pre-heated humidity chamber.

3. Primary antibody incubation

3.1 Use the provided **Primary Antibody Diluent** (1x) to dilute the **Mouse and Rabbit Primary Antibodies** according to the table below.

Antibody/ Diluent	Volume
Mouse anti-Her2 antibody (100x)	1 µl
Rabbit anti-Her2 antibody (100x)	1 µl
Naveni Primary Antibody Diluent	98 µl
Total:	100 µl

3.2 Decant the Blocking Buffer and add enough of the antibodies to cover one cell pellet – add 40 µl of Primary Antibody Diluent to the second cell pellet as negative control.

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. Probe incubation	4.1	Prepare the probes by diluting **Probe M1** and **Probe R2** in **Probe Diluent** (1x) (dilute 1:40 each).
	4.2	Add enough of the probes to cover the sample area.
	4.3	Incubate for 60 min at +37 °C in a pre-heated humidity chamber.
	4.4	Decant the solution and wash slides for 3x5 min with 1x TBS-T in a staining jar under gentle agitation.
5. Reaction A	5.1	Start preparing **Reaction A** by diluting **Buffer A** (5x) 1:5 in water. Vortex and spin down.
	5.2	Add **Enzyme A** (dilute 1:40) Mix gently by pipetting and spin down.
	5.3	Add enough Reaction A to cover the sample area.
	5.4	Incubate for 60 min at +37 °C in a pre-heated humidity chamber.
	5.5	Decant the solution and wash slides for 2x3 min with 1x TBS-T in a staining jar under gentle agitation.
6. Reaction B	6.1	Start preparing **Reaction B** by diluting **Buffer B** (5x) 1:5 in water. Vortex and spin down.
	6.2	Add **Enzyme B** (dilute 1:40). Mix gently by pipetting and spin down.
	6.3	Add enough Reaction B to cover the sample area.
	6.4	Incubate for 30 min at 37 °C in a pre-heated humidity chamber.
	6.5	Wash slides for 2x3 min with 1x TBS-T in a staining jar under gentle agitation.
7. Reaction C	7.1	Select the **Buffer C** vial with the appropriate detection fluorophore for your microscope filter set. Do not use more than one **Buffer C** vial.
Protect from light	7.2	Start preparing **Reaction C** by diluting **Buffer C** (5x) 1:5 in water. Vortex and spin down.
	7.3	Add **Enzyme C** (dilute 1:40). Mix gently by pipetting and spin down.
	7.4	Add enough Reaction C to cover the sample area.
	7.5	Incubate for 90 min at +37 °C in a pre-heated humidity chamber.
	7.6	Decant the solution and wash slides for 2 min with 1x TBS in a staining jar under gentle agitation.

8. Nuclei staining and mounting (not provided)	8.1	Start preparing a Nuclei staining solution by diluting Dapi in 1xPBS. Vortex and spin down.
	8.2	Decant wash buffer from the slides.
	8.3	Add enough Nuclei staining solution to cover the sample area.
Protect from light	8.4	Incubate for 5 min at room temperature on the bench.
	8.5	Decant the solution and wash slides for 2x 10 min with 1x TBS in a staining jar under gentle agitation.
	8.6	Wash slides for 15 min with 0.1x TBS in a staining jar under gentle agitation.
9. Mounting (not provided)	9.1	Decant excess wash buffer from the slides.
	9.2	Mount the slides with a coverslip using an anti-fade mounting medium.
Protect from light	9.3	Image your slides in fluorescence or confocal microscope, using 20x objective or higher and filter set for DAPI and a corresponding fluorophore (FITC for Atto 488, Texas Red, or Cy5 for Atto 647, respectively).

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

RECOMMENDED IMAGING SETTINGS:

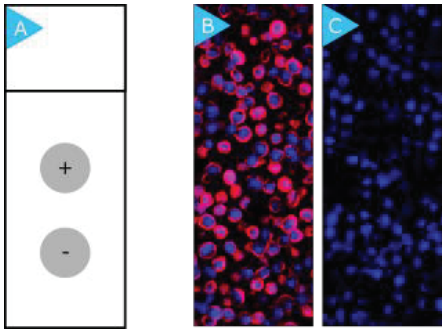

Fig. 1: The recommended slide layout, where "**+**" indicates both primary antibodies present whilst "**–**" indicates no primary antibodies present (**A**). Reference images displaying a typical positive (**B**) and negative (**C**) result.