NaveniFlex Cell

Protocol for 96-well half area plates

Instructions For Use

For research use only

Manufactured by:

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General guidelines

- The volumes in this protocol are optimized for 96-well half area plates.
- Reaction volume depends on sample size and should be sufficient to cover the entire sample area. A volume of approximately 40 µl/cm^2 is recommended.
- Use best practices when pipetting to minimize reagent consumption.
- Thoroughly defrost all buffers at room temperature. Vortex well before use.
- Centrifuge vials before pipetting.
- Keep enzymes on ice or on a frozen cooling block.
- Spin down Naveni Enzyme 1 (40x) and Naveni Enzyme 2 (40x) before use.
- Add enzymes immediately before adding the reaction mix to the sample.
- The recommended Navenibody working dilution is 1:40. Depending on target abundance, Navenibodies can be titrated down to 1:80.
- Aspirate wash buffer and reaction solutions as much as possible from the samples between each step. It is recommended to invert and tap on absorbent paper to remove excess liquid.
- Unused solutions should be disposed of according to local regulations.
- Use a plate lid or seal the plates to prevent evaporation of reaction solutions during incubations.
- Incubation times or assay temperatures other than those specified in the instructions for use may negatively impact the results. Warm washing buffer is only required for step 4.4. Additional warm washing steps may lead to signal deterioration.
- 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.

Instructions for use

1. Pre-treatment (reagents not provided)

1.1. Seed the cells of interest at the desired density in a 96-well half area plate and let the cells adhere according to the appropriate cell protocol.
1.2. Fix and permeabilize cells according to the immunofluorescence protocol optimized for the primary antibodies utilized in this assay.
1.3. Prepare 1x TBS-T* and pre-warm enough buffer to 37 °C to perform Step 4.4 below.

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

2. Blocking

2.1. Add Naveni Block to the wells to completely cover the 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 Naveni Diluent to dilute your primary antibodies to a working concentration. Start with a concentration optimized for/recommended by the antibody vendor for ICC.
3.2. Aspirate the Naveni Block and add a sufficient volume of the antibody working solution from step 3.1 to cover the sample area.
3.3. Seal the plate or use a plate lid and incubate for 60 min at 37 °C, or at 4 °C overnight.
3.4. Aspirate the antibody solution and add 150 µL of 1x TBS-T to each well. Repeat three times. For the last wash, incubate for 15 min at room temperature before aspiration. Invert and tap on absorbent paper to remove excess liquid.

4. Navenibody incubation

4.1. Dilute Navenibody M1 and Navenibody R2 1:40 in Naveni Diluent.
4.2. Add a sufficient volume of the Navenibody working solution from step 4.1 to cover the sample area.
4.3. Seal the plate or use a plate lid and incubate for 60 min at 37 °C.
4.4. Aspirate the Navenibody solution and add 150 µL of pre-warmed TBS-T from step 1.3 to each well. Aspirate the wash buffer and repeat the procedure three times. For the last wash, incubate for 15 min at room temperature before aspiration. Invert and tap on absorbent paper to remove excess liquid.

5. Reaction 1

5.1. Start preparing Reaction 1 by diluting Naveni Buffer 1 (5x) 1:5 in water. Vortex and spin down.
5.2. Add Naveni Enzyme 1 (40x) so that it is diluted 1:40. Mix gently by pipetting and spin down. See **table 1** for an example calculation.
5.3. Add a sufficient volume of Reaction 1 to cover the sample area.
5.4. Seal the plate or use a plate lid and incubate for 60 min at 37 °C.
5.5. Aspirate the solution and add 150 µL of 1x TBS-T to each well. Aspirate the wash buffer and repeat the procedure three times. For the last wash, incubate for 5 min at room temperature. Invert and tap on absorbent paper to remove excess liquid.

Table 1: Example calculation for a 40 µL reaction volume.

Reagent for 1 cm^2 sample area	Volume
H_2O	31 µL
Naveni Buffer 1 (5x)	8 µL
Naveni Enzyme 1 (40x)	1 µL
Total volume:	40 µL

6. Reaction 2 (protect from light!)

6.1. Start preparing Reaction 2 by diluting NaveniFlex Cell Buffer 2 Red (5x) or NaveniFlex Cell Buffer 2 Atto647N (5x) 1:5 in water. Vortex and spin down.
6.2. Add Naveni Enzyme 2 (40x) 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 in the wells.
6.4. Seal the plate or use a plate lid and incubate for 90 min at 37° C.
6.5. Aspirate the solution and add 150 µL of 1x TBS to each well. Aspirate the wash buffer and repeat one time. For the last wash, incubate for 3 min at room temperature. Invert and tap on absorbent paper to remove excess liquid[1].

7. Nuclear stain and mounting (reagents not provided; protect from light!)

7.1. Add DAPI or a nuclear stain of choice with a similar emission spectrum. Dilute and incubate as recommended by the vendor.
7.2. Aspirate the solution and add 150 µL of TBS in each well and incubate for 5 minutes at room temperature. Aspirate the wash buffer and repeat the wash one time.
7.3. Add 50 µL of sterile filtered 1x PBS to each well.

8. Imaging

8.1. Image the plates with a fluorescent plate reader with the desired objective.
8.2 For imaging, a filter set corresponding to Cy3.5/TexasRed filter set (Ex: 585-595 nm, Em: 615-625nm), Cy5 filter set (Ex: 635-645 nm; Em 665-675 nm) and DAPI filter set is required.

[1]Fluorescently labelled primary antibodies may be diluted in Diluent 1 and added after this step if co-staining is desired. Dilute and incubate as recommended by the manufacturer.

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