PPI.TCR01.FR.100

Naveni® CD8/MHC-I Atto647N

General guidelines

- We recommend using a pressure cooker for the antigen retrieval in step 1.1. If using a different method, longer time can be needed for successful antigen retrieval.
- Reaction volume depends on sample size and should be sufficient to cover the entire sample area. A volume of approximately 40 µL/cm² is recommended. For example, for an FFPE tissue section that covers an area of 5 cm² on a slide, we recommend using a reaction volume of 200 µL/slide.
- Use best practices when pipetting to minimize reagent consumption.
- Thoroughly defrost all buffers at room temperature. Vortex well before use.
- Centrifuge vials before pipetting.
- Gently mix and spin down Enzymes 1 and 2 before use.
- Keep enzymes on ice or on a frozen cooling block.
- Add enzymes right before adding reaction mix to the sample.
- Preheat humidity chambers before each step.
- Incubation times or assay temperature other than those specified may negatively impact results. A warm washing buffer is only required for step 4.5. Additional warm washing steps may lead to signal deterioration.
- Consider fluorophore compatibility and bleed-through if performing an immunofluorescence co-stain.
- The Detection Reagent is light sensitive. Always keep it protected from light.
- Remove excess washing buffer from samples before adding the reagent mix.
- The Naveni® CD8/MHC-I kit can be combined with standard immunofluorescence (IF), provided that all primary antibodies are either raised in different species, and/or labelled with fluorophores different from the ones used in the kit.
- Do not allow slides/samples to dry out.
- 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.
- Unused solutions should be disposed of according to local regulations.

Additional reagents, not provided

- Tris-EDTA antigen retrieval buffer (pH 8.5)
- Wash buffers
- DAPI or other nuclear stain
- Anti-fade mounting medium

Important:

Appropriate precautions should be taken to avoid antibody cross-contamination between samples. Cross-contamination is the primary source of unspecific background due to the high sensitivity of the assay.

Avoid bulk washing methods when using different primary antibody pairs or when washing different technical controls.

Kit components

Box 1.1:

Storage: 4 to 8°C

DO NOT FREEZE

Material	Art.no	Amount
Block	NT.1.100.01	4 mL
Diluent	NB.1.100.02	2X 8 mL
Navenibody M1 (80X)	NB.1.100.06	100 µL
Navenibody R2 (80X)	NB.1.100.07	100 µL

Box 2:

Storage: -25 to -15°C. Protect from light!

FREEZE

Material	Art.no	Amount
Buffer 1 (5X)	NB.2.100.17	800 µL
Enzyme 1 (40X)	NF.2.100.11	100 µL
Buffer 2 (5X)	NT.2.100.01	800 µL
Enzyme 2 (40X)	NF.2.100.15	100 µL
Post-Block Supplement (40X)	NT.2.100.04	100 µL
Detection Mix (5X), Atto647N	NT.2.100.06	800 µL
CD8 Antibody (200X) based on clone SP239, Abcam	PPI.2.03	20 µL
MHC-I Antibody (200X)	PPI.2.04	20 µL

When stored as directed, the product is stable for at least 3 months after receipt.

Instructions for use

1. Pre-treatment and wash buffer preparation (reagents not provided)

1.1. Deparaffinize samples and perform heat induced epitope retrieval with Tris-EDTA buffer (pH 8.5) at a recommended temperature of 110°C for 10 min.
1.2. Where applicable, use a hydrophobic barrier pen around the sample to prevent spillage and sample drying.
1.3. Prepare two bottles of 1X Tris-buffered saline supplemented with 0.05% Tween 20) (TBS-T). Pre-warm one bottle to 37°C and the required number of staining jars. Warm wash is required after Navenibody incubation at step 4.5.

2. Blocking

2.1. Add Block solution to the entire sample area.
2.2. Incubate for 60 min at 37°C in a preheated humidity chamber.

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

3. Primary antibody incubation

3.1. Use the provided Diluent to dilute the primary CD8 and MHC-I antibodies 1:200 to the working concentration. See Table 1 for an example calculation for a total reaction volume of 200 µL.
3.2. Decant the Block from the slides and add a sufficient volume of the antibody working solution prepared in step 3.1 to cover the sample area.
3.3. Incubate slides overnight at 4°C in a humidity chamber.
3.4. Decant the antibody solution.
3.5. Rinse slides 2x 10 sec and 1x 15 min with 1X TBS-T in a staining jar and gentle agitation. Wash controls in a separate staining jar.

Table 1: Example calculation

Reagent for 5 cm^2 sample area	Volume (µL)
Diluent (1X)	198
CD8 antibody (200X) clone SP239	1
MHC-I antibody (200X)	1
Total volume:	200 µL

4. Navenibody incubation

4.1. Dilute Navenibody M1 and Navenibody R2 1:80 each in the provided Diluent.
4.2. Add a sufficient volume of the Navenibody working solution from step 4.1 to cover the sample area.
4.3. Incubate for 60 min at 37 °C in a preheated humidity chamber.
4.4. Decant the Navenibody solution from the slides.
4.5. Wash slides 2x 10 sec and 1x 15 min with pre-warmed 1X TBS-T in the staining jar from step 1.3 and gentle agitation. Wash controls separately.

5. Reaction 1

For an example calculation sufficient for a sample area of ~5 cm^2, see Table 2.
5.1. Dilute Buffer 1 1:5 in distilled water. Use the total Reaction 1 volume to determine the amount of distilled water required for the dilution. Vortex and spin down.

Table 2: Example calculation

Reagent for 5 cm^2 sample area	Volume (µL)
dH_2O	155
Buffer 1 (5x)	40
Enzyme 1 (40x)	5
Total volume:	200 µL

5.2. Add Enzyme 1 1:40 to the reaction mix prepared in step 5.1. Mix gently by pipetting and spin down.
5.3. Add a sufficient volume of Reaction 1 to cover the sample area.
5.4. Incubate for 30 min at 37 °C in a preheated humidity chamber.
5.5. Decant Reaction 1.
5.6. Rinse the slides with TBS-T to remove the excess of Reaction 1 and then wash 1x 5 min with 1X TBS-T in a staining jar and gentle agitation.

6. Reaction 2

For an example calculation sufficient for a sample area of ~5 cm^2, see Table 3.
6.1. Dilute Buffer 2 1:5 in distilled water. Use the total Reaction 1 volume to determine the amount of distilled water required for the dilution. Vortex and spin down.

Table 3: Example calculation

Reagent for 5 cm^2 sample area	Volume (µL)
dH_2O	155
Buffer 2 (5x)	40
Enzyme 2 (40x)	5
Total volume:	200 µL

6.2. Add Enzyme 2 1:40 to the reaction mix prepared in step 6.1. Mix gently by pipetting and spin down.
6.3. Add a sufficient volume of Reaction 2 to cover the sample area.
6.4. Incubate for 90 min at 37°C in a preheated humidity chamber. Proceed to step 7.1 before the incubation is over.

7. Post-block

7.1. While Reaction 2 is incubating, thaw and dilute Post-Block Supplement 1:40 in Diluent. Vortex and spin down.
7.2. After the incubation step 6.4 is complete, decant Reaction 2 and add a sufficient volume of the Post-Block working solution from step 7.1 to cover the sample area.
7.3. Incubate for 30 min at 37°C in a preheated humidity chamber.

8. Detection. Protect from light!

8.1. While the Post-Block is incubating, thaw and prepare the Detection working solution by diluting Detection Mix 1:5 in water. Vortex and spin down.
8.2. Optional: Fluorescently labelled primary antibodies may be added together with the detection working solution if co-staining is desired. Dilute and incubate as recommended by the manufacturer.
8.3. Decant the Post-Block Supplement solution. Do not wash at this step, but make sure the Post-block solution has been removed. Add a sufficient volume of Detection working solution from step 8.1 to cover the sample area.
8.4. Incubate for 30 min at 37°C in a preheated humidity chamber.
8.5. Decant the Detection solution and wash slides with TBS for 2 min within a staining jar and gentle agitation.

9. Nuclear stain and mounting (reagents not provided) Protect from light!

9.1. Decant washing buffer from the slides.
9.2. Add DAPI or a nuclear stain of choice with a similar emission spectrum diluted in PBS to a concentration recommended by the vendor. Incubate for 5 min at room temperature in a humidity chamber.
9.3. Decant the nuclear stain solution, wash slides 2x5 min in 1X TBS and gentle agitation.
9.4. Perform a final 5 min wash in 0.1X TBS under gentle agitation.
9.5. Dry slides in a slide centrifuge or by air-drying. Mount slides with a coverslip using an antifade mounting medium.

10. Imaging

10.1. Image your slides on a fluorescence or confocal microscope using a 20x objective or higher.
10.2. For imaging, a filter set corresponding to your chosen nuclear stain and detection fluorophores' excitation/emission spectra are required (Table 4).

Table 4: Filter sets for imaging.

Filter set	Detecting	λ Excitation	λ Emission
DAPI	Nuclei		
FITC	IF (Optional)	480-490 nm	525-535 nm
Cy3	IF (Optional)	545-555 nm	575-585 nm
Texas Red	IF (Optional)	585-595 nm	615-625 nm
Cy5	CD8/MHC-I interaction by Atto647 Detection Mix	635-645 nm	665-675 nm

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