Art.no 60034

Naveni® PD1/PD-L1 Atto647N

Instructions For Use

For research use only

Manufactured by:

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General guidelines

- 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 you use 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.
- Spin down Naveni Enzyme 1 (40x) and Naveni Enzyme 2 (40x) before use.
- Keep enzymes on ice or on a frozen cooling block.
- Add enzymes right before adding reaction mix to sample.
- Detection reagent is light-sensitive. Always keep it protected from light.
- Remove excess washing buffer from samples before adding the reagent mix.
- Do not allow slides/samples to dry.
- Preheat humidity chamber before each step.
- Incubation times or assay temperatures other than those specified may negatively impact 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.
- Unused solutions should be disposed of according to local regulations.

Additional reagents, not provided

- Antigen Retrieval Buffer pH 9.0 (10x), *Navinci 60058*
- Wash buffers
- DAPI or other nuclear stain
- Mounting medium

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 different primary antibody pairs are used, or when washing different technical controls.

Kit components

Box 1:

Art. no. 60014
Storage: 2 to 8°C

DO NOT FREEZE

Material	Art.no	Amount
Naveni Block	50014	6 mL
Naveni Diluent	50001	2x 8 mL
Navenibody M1	50049	100 µL
Navenibody R2	50051	100 µL

Box 2:

Art. no. 60023
Storage: -25 to -15°C. Protect from light!

FREEZE

Material	Art.no	Amount
Naveni Buffer 1 (5x)	50018	1.3 mL
Naveni Enzyme 1 (40x)	50002	100 µL
NaveniFlex Tissue Buffer 2 (5x)	50022	800 µL
Naveni Enzyme 2 (40x)	50003	100 µL
Naveni Post-Block (40x)	50006	100 µL
Naveni Detection Mix (5x), Atto647N	50038	1300 µL

Bag 1:

Art. no. 60043
Storage: -25 to -15°C. Protect from light!

FREEZE

Material	Art.no	Amount
Naveni PD1 Antibody	50041	100 µL
Naveni PD-L1 Antibody	50042	100 µL

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Instructions for use

1. Pre-treatment

The method for pre-treatment of tissue sections is described in the Instructions For Use of Antigen Retrieval Buffer pH 9.0 (10x), Navinci, 60058, IFU-0032.

2. Preparation of warm buffers

2.1. Prepare two bottles of 1x TBS-T*, pre-warm one bottle to 37°C together with required number of staining jars (warm wash is required after Navenibody incubation at Step 5.4).

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

3. Blocking

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

4. Primary antibody incubation

4.1. Use the provided Naveni Diluent to dilute Naveni PD1 Antibody and Naveni PD-L1 Antibody down to working concentration (dilute each antibody 1:40) (**See table 1**).

Table 1: Example calculation for a total reaction volume of 200 µL, sufficient for a sample area of ~5 cm².

Reagent for 5 cm² sample area	Volume
Naveni Diluent	190 µL
Naveni PD1 Antibody	5 µL
Naveni PD-L1 Antibody	5 µL
Total volume:	200 µL

4.2. Decant the block solution from the slides and add sufficient volume of the antibody working solution from step 4.1 to cover the sample area.
4.3. Incubate for 60 min at 37 °C or overnight at 4 °C in a humidity chamber.
4.4. Decant the antibody solution, wash slides 2x 10 sec and 1x 15 min with 1x TBS-T in a staining jar under gentle agitation. Wash controls separately.

5. Navenibody incubation

5.1. Dilute Navenibody M1 and Navenibody R2 1:80 in the provided Naveni Diluent.
5.2. Add a sufficient volume of the Navenibody working solution from step 5.1 to cover the sample area.
5.3. Incubate for 60 min at 37 °C in a preheated humidity chamber.
5.4. Decant the solution and wash slides with the pre-warmed 1x TBS-T from Step 2.1. Wash for 2x 10 sec and 1x 15 min in the pre-warmed staining jar under gentle agitation. Wash controls separately.

6. Reaction 1

6.1. Start preparing Reaction 1 by diluting Naveni Buffer 1 (5x) 1:5 in water. Vortex and spin down.
6.2. Add Naveni Enzyme 1 (40x) so that it is diluted 1:40. Mix gently by pipetting and spin down.

Table 2: Example calculation for a total reaction volume of 200 µL, sufficient for a sample area of ~5 cm².

Reagent for 5 cm² sample area	Volume
H_2O	155 µL
Naveni Buffer 1 (5x)	40 µL
Naveni Enzyme 1 (40x)	5 µL
Total volume:	200 µL

6.3. Add a sufficient volume of Reaction 1 to cover the sample area.
6.4. Incubate for 30 min at 37 °C in a preheated humidity chamber.
6.5. Decant the solution, wash slides 1x 10 sec and 1x 5 min with 1x TBS-T in a staining jar under gentle agitation.

7. Reaction 2

7.1. Start preparing Reaction 2 by diluting NaveniFlex Tissue Buffer 2 (5x) 1:5 in water. Vortex and spin down.
7.2. Add Naveni Enzyme 2 (40x) so that it is diluted 1:40. Mix gently by pipetting and spin down.
7.3. Add a sufficient volume of Reaction 2 to cover the sample area.
7.4. Incubate for 90 min at 37 °C in a preheated humidity chamber. Proceed to step 8.1 before the incubation is over.

8. Post-block

8.1. While Reaction 2 is incubating, thaw and dilute Naveni Post-Block (40x) 1:40 in Naveni Diluent. Vortex and spin down.
8.2. After the incubation step is complete, decant Reaction 2 and add a sufficient volume of the post-block working solution from step 8.1 to cover the sample area.
8.3. Incubate for 30 min at 37 °C in a preheated humidity chamber.

9. Detection: protect from light!

9.1. Prepare the detection working solution by diluting Naveni Detection mix (5x) Atto647N 1:5 in water. If desired, it is possible to add fluorescent (FITC or Cy3) labeled antibodies for co-staining at this step.
9.2. Decant the post-block solution from the slides. 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 9.1 to cover the sample area.
9.3. Incubate for 60 min at 37 °C in a preheated humidity chamber.
9.4. Decant the solution and wash slides for 2 min with 1x TBS-T in a staining jar under gentle agitation.

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

10.1. Remove excess washing buffer from the slides.
10.2. Add DAPI or a nuclear stain of your 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.
10.3. Decant the solution, wash slides 2x 5 min in 1x TBS-T under gentle agitation.
10.4. Perform a final 5 min wash in 0.1x TBS-T under gentle agitation. Dry slides in a slide centrifuge or air-dry them and mount with a coverslip using an antifade mounting medium.

11. Imaging

11.1. Image your slides on a fluorescence or confocal microscope using a 20x objective or higher.
11.2. For imaging, a filter set corresponding to your chosen nuclear stain and detection fluorophore's excitation/ emission spectra is required, see **table 3**.

Table 3: Filter sets for imaging.

Filter set	Detecting	λ Excitation	λ Emission
DAPI	Nuclei		
Cy5	PD1/PD-L1 interaction	635-645 nm	665-675 nm

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