Art.no 60033

NaveniBright™ PD1/PD-L1

Instructions For Use

For research use only

Manufactured by:

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General guidelines

- Do not mix NaveniBright PD1/PD-L1 reagents with other Naveni® product lines.
- 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 that 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.
- Gently mix and 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 applying reaction mix to sample.
- Preheat humidity chamber before each step.
- Incubation times or assay temperatures other than those specified may negatively impact results.
- All washing steps are performed on a platform shaker with gentle agitation.
- Warm washing buffer is only required for step 4.5, additional warm washing steps may lead to signal deterioration.
- Remove excess washing buffer from samples before adding the reagent mix.
- Do not allow slides/samples to dry.
- 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.
- Chromogens may be carcinogenic and should be handled with care.

Additional reagents, not provided

- Antigen Retrieval Buffer pH 9.0 (10x), *Navinci 60058*
- Endogenous alkaline phosphatase/ horseradish peroxidase blocking solution.
- Tris-buffered saline (TBS) and Tris-buffered saline supplemented with 0.05% Tween (TBS-T).
- VectaMount® Express Mounting Medium (H-5700-60) from Vector Laboratories.

Application

NaveniBright PD1/PD-L1 is an *in situ* proximity ligation assay for the study of PD1 and PD-L1 interaction in formalin-fixed paraffin-embedded human tissues and cells samples. For research use only. Not for use in diagnostic procedures.

Detection enzyme and substrate

- Horseradish peroxidase
- Red precipitating reaction product

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 multiple antibodies are used.

Kit components

Box 1:

Art.no. 60036
Storage: 2 to 8°C

DO NOT FREEZE

Material	Art.no	Amount
NaveniBright Block	50000	6 mL
Naveni Supplement 1	50027	700 µL
Naveni Diluent	50015	15 mL
Naveni Supplement 2	50028	1.3 mL
Naveni HRP Diluent	50016	8 mL
Navenibody M1	50049	100 µL
Navenibody R2	50051	100 µL
Naveni HRP Reagent	50034	30 µL
Naveni HRP Substrate 1	50029	180 µL
Naveni HRP Substrate 2	50030	120 µL
Naveni HRP Substrate 3	50031	120 µL
Naveni HRP Substrate 4	50032	180 µL

Bag 1:

Art. no. 60038
Storage: 2 to 8°C

DO NOT FREEZE

Material	Art.no	Amount
NaveniBright Nuclear Stain	50056	6 mL

Bag 2:

Art. no. 60043
Storage: -25 to -15°C

FREEZE

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

Box 2:

Art. no. 60045
Storage: -25 to -15°C.

FREEZE

Material	Art.no	Amount
Naveni Buffer 1 (5x)	50018	1.3 mL
Naveni Enzyme 1 (40x)	50002	100 µL
NaveniBright Buffer 2 (5x)	50023	800 µL
Naveni Enzyme 2 (40x)	50003	100 µL

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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. Wash slides for 2x5 min in 1x TBS-T.
2.2. Prepare two bottles of 1x TBS-T* and pre-warm one bottle to 37°C together with the required number of staining jars (warm wash is required after Navenibody incubation at Step 5.4).

3. Blocking

3.1. Prepare blocking solution by adding 5 µL of Naveni Supplement 1 to every 40 µL of NaveniBright Block (**table 1**).
3.2. Add the prepared blocking solution from step 2.1 to the entire sample area (approximately 40 µL for each 1 cm² area).
3.3. Incubate for 60 min at 37 °C in a preheated humidity chamber.

4. Primary antibody incubation

4.1. Prepare primary antibody solution by adding 5 µL of Naveni Supplement 2 to every 40 µL of Naveni Diluent (**table 1**).
4.2. Use the prepared primary antibody solution from step 4.1 to dilute Naveni PD1 Antibody and Naveni PD-L1 Antibody to 1x (dilute 1:40 each).
4.3. Decant the blocking solution from slides and wash for 2x 3 min in 1x TBS-T.
4.4. Add enough of the diluted antibodies to cover the sample area.
4.5. Incubate for 60 min at 37 °C or overnight at +4 °C in a humidity chamber.
4.6. Decant the antibody solution and wash slides for 3x 5 min in 1x TBS-T in a staining jar.

Table 1: Blocking solution and primary antibody solution.

Kit component	Blocking solution	Primary antibody solution
NaveniBright Block	40 µL	-
Naveni Supplement 1	5 µL	-
Naveni Diluent	-	40 µL
Naveni Supplement 2	-	5 µL
Total volume:	45 µL	45 µL

5. Navenibody incubation

5.1. Dilute Navenibody M1 and Navenibody R2 1:40 in Naveni Diluent.
5.2. Add 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 Navenibody solution.
5.5. Wash slides for 2x 10 sec and 1x 15 min with the pre-warmed 1x TBS-T in the staining jar from step 2.2. Wash the controls separately.

6. Reaction 1

6.1. Dilute Naveni Buffer 1 (5x) 1:5 in distilled water. Vortex and spin down.
6.2. Prepare Reaction 1 by adding Naveni Enzyme 1 (40x) 1:40 to the diluted buffer from step 6.1. Mix gently by pipetting and spin down.
6.3. Add 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. Wash slides for 2x 3 min in 1x TBS-T in a staining jar.

7. Reaction 2

7.1. Dilute NaveniBright Buffer 2 (5x) 1:5 in distilled water. Vortex and spin down.
7.2. Prepare Reaction 2 by adding Naveni Enzyme 2 (40x) 1:40 to the diluted buffer from step 7.1. Mix gently by pipetting and spin down.
7.3. Add sufficient volume of Reaction 2 to cover the sample area.
7.4. Incubate for 90 min at 37 °C in a preheated humidity chamber.

8. HRP incubation

8.1. Decant the solution and wash slides for 2x 5 min in 1x TBS, followed by 1x 10 min in 0,1x TBS in a staining jar.
8.2. Dilute the Naveni HRP Reagent 1:800 in Naveni HRP Diluent.
8.3. Decant wash buffer from the slides.
8.4. Add sufficient volume of HRP solution to cover the sample area.
8.5. Incubate for 30 min at room temperature with slow agitation on a platform shaker.

9. Substrate development

9.1. Decant the solution and wash slides for 2x 2 min in 1x TBS in a staining jar.
9.2. Prepare the substrate solution by mixing Naveni HRP Substrate 1 (dilute 62.5x), Naveni HRP Substrate 2 (dilute 100x), Naveni HRP Substrate 3 (dilute 100x) and Naveni HRP Substrate 4 (dilute 62.5x) in distilled water +.
For an example calculation for a minimal reaction volume, see **table 2**.

Table 2: Example calculation.

Kit Component	Substrate Solution (µL)
Distilled water	94.8 µL
Naveni HRP Substrate 1	1.6 µL
Naveni HRP Substrate 2	1.0 µL
Naveni HRP Substrate 3	1.0 µL
Naveni HRP Substrate 4	1.6 µL
Total:	100 µL

9.3. Decant wash buffer from the slides.
9.4. Add first substrate solution to cover the sample area.
9.5. Incubate the slides with substrate at room temperature for 5 to 10 min. ++
9.6. Decant of the substrate solution from the slides and wash slides for 2x 2 min in deionized water.

10. Nuclear staining

10.1. Decant wash buffer from the slides.
10.2. Add enough NaveniBright Nuclear Stain to cover the sample area.
10.3. Incubate for 2 to 10 seconds at room temperature. +++
10.4. Rinse the slides under running tap water (not deionized water).

11. Dehydration and mounting ¤

11.1. Wash slides in water for 5 min.
11.2. Rapid dehydrate slides with 2x 1 min wash in isopropanol.
11.3. Blot excess isopropanol from slides and apply VectaMount® Express Mounting Medium (H-5700-60).
11.4. Apply the coverslips and allow slides to dry flat at room temperature for 10 to 20 min.
11.5. Analyze using a brightfield microscope, using at least a 20x objective.
11.6. After imaging, store the slides at room temperature. The signal is stable for years.

* TBS-T (Tris-buffered saline supplemented with 0,05% Tween 20).
+ For alternative substrates, prepare according to manufacturers' user guide.
++ Substrate incubation time should be optimized for each assay.
+++ Excessive nuclear staining may obscure developed signals.
¤ Slides must be mounted with VectaMount® Express Mounting Medium (H-5700-60) from Vector Laboratories. Usage of other dehydration methods or mounting medium may lead to reduced signal intensity.

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