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.
- 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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Instructions for use

1. Pre-treatment

1.1. Perform the steps described in the Instructions For Use of Antigen Retrieval Buffer pH 9.0 (10x), Navinci, 60058, IFU-0032.
1.2. After antigen retrieval, add enough alkaline phosphatase/ horseradish peroxidase blocking solution (for quenching; not provided) to cover each sample. Incubate according to manufacturers' user guide.
1.3. Wash slides for 2x5 min in 1x TBS-T.

2. Preparation of warm buffers

2.1. 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 2.1. 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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