

Naveni® PD1/PD-L1 AP

General guidelines

- Do not mix Naveni® 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 Enzymes 1 and 2 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.
- VectaMount® Express Mounting Medium (H-5700-60) from Vector Laboratories.

Application

Naveni®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 1.1:

Storage: 4 to 8°C



Material	Art.no	Amount
Blocking Buffer (1x)	NB.1.100.01	4000 µL
Supplement 1	NB.1.100.03	500 µL
Antibody Diluent (1x)	NB.1.100.02	8000 µL
Supplement 2	NB.1.100.04	1000 µL
Navenibody Diluent (1x)	NB.1.100.02	4000 µL
Navenibody M1 (40x)	NB.1.100.06	100 µL
Navenibody R2 (40x)	NB.1.100.07	100 µL

Box 1.2:

Storage: 4 to 8°C



Material	Art.no	Amount
AP Diluent	NB.1.100.08	8000 µL
AP Reagent	NB.1.100.10	100 µL
AP Substrate Diluent	NB.1.100.09	8000 µL
AP Substrate 1	NB.1.100.11	140 µL
AP Substrate 2	NB.1.100.12	100 µL

Box 1.3:

Storage: 4 to 8°C



Material	Art.no	Amount
Nuclear Stain	NB.1.100.16	6000 µL

Box 2:

Storage: -25 to -15°C.



Material	Art.no	Amount
PD1 antibody (40x) based on clone EH33 CST	PPI.2.01	100 µL
PD-L1 antibody (40x) based on clone SP142 Abcam RabMAb®	PPI.2.02	100 µL
Buffer 1 (5x)	NB.2.100.17	800 µL
Enzyme 1 (40x)	NF.2.100.11	100 µL
Buffer 2 (5x)	NB.2.100.18	800 µL
Enzyme 2 (40x)	NF.2.100.15	100 µL

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



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

Instructions for use

1. Sample preparation

- 1.1. 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.
- 1.2. Wash slides for 2x5 min in 1x TBS-T.
- 1.3. 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 4.5).

2. Blocking

- 2.1. Prepare blocking solution by adding 5 µL of **Supplement 1** to every 40 µL of **Blocking Buffer (1x)** (table 1).
- 2.2. Add the prepared blocking solution from step 2.1 to the entire sample area (approximately 40 µL for each 1 cm² area).
- 2.3. Incubate for 60 min at 37 °C in a preheated humidity chamber.

3. Primary antibody incubation

- 3.1. Prepare primary antibody solution by adding 5 µL of **Supplement 2** to every 40 µL of **Antibody Diluent (1x)** (table 1).
- 3.2. Use the prepared primary antibody solution from step 3.1 to dilute **PD1** and **PD-L1 antibody** to 1x (dilute 1:40 each).
- 3.3. Decant the blocking solution from slides and wash for 2x 3 min in 1x TBS-T.
- 3.4. Add enough of the diluted antibodies to cover the sample area.
- 3.5. Incubate for 60 min at 37 °C or overnight at +4 °C in a humidity chamber.
- 3.6. 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
Blocking Buffer	40 µL	-
Supplement 1	5 µL	-
Antibody diluent	-	40 µL
Supplement 2	-	5 µL
Total volume:	45 µL	45 µL

4. Navenibody incubation

- 4.1. Dilute **Navenibody M1** and **Navenibody R2** 1:40 in **Navenibody Diluent (1x)**.
- 4.2. Add 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.
- 4.5. Wash slides for 2x 10 sec and 1x 15 min with the pre-warmed 1x TBS-T in the staining jar from step 1.3. Wash the controls separately.

5. Reaction 1

- 5.1. Dilute **Buffer 1** 1:5 in distilled water. Vortex and spin down.
- 5.2. Prepare **Reaction 1** by adding **Enzyme 1** 1:40 to the diluted buffer from step 5.1. Mix gently by pipetting and spin down.
- 5.3. Add 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. Wash slides for 2x 3 min in 1x TBS-T in a staining jar.

6. Reaction 2

- 6.1. Dilute **Buffer 2** 1:5 in distilled water. Vortex and spin down.
- 6.2. Prepare **Reaction 2** by adding **Enzyme 2** 1:40 to the diluted buffer from step 6.1. Mix gently by pipetting and spin down.
- 6.3. Add sufficient volume of **Reaction 2** to cover the sample area.
- 6.4. Incubate for 90 min at 37 °C in a preheated humidity chamber.

7. AP incubation

- 7.1. 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.
- 7.2. Dilute the **AP reagent** 1:300 in **AP diluent**.
- 7.3. Decant wash buffer from the slides.
- 7.4. Add sufficient volume of AP solution to cover the sample area.
- 7.5. Incubate for 30 min at room temperature with slow agitation on a platform shaker.

8. Substrate development

- 8.1. Decant the solution and wash slides for 2x 2 min in 1x TBS in a staining jar.
- 8.2. Prepare the substrate solution by mixing **AP Substrate 1** (dilute 62,5x) and **AP Substrate 2** (dilute 80x) in **AP Substrate Diluent** +.

For an example calculation for a minimal reaction volume see table 2.

Table 2: Example calculation.

Kit Component	Substrate solution (µL)
AP Substrate Diluent	80 µL
AP Substrate 1	1,3 µL
AP Substrate 2	1,0 µL
Total:	82,3 µL

- 8.3. Decant wash buffer from the slides.
- 8.4. Add first substrate solution to cover the sample area.
- 8.5. Incubate the slides with substrate at room temperature for 25 min. ++
- 8.6. Decant of the substrate solution from the slides and wash slides for 2x 2 min in deionized water.

9. Nuclear staining

- 9.1. Decant wash buffer from the slides.
- 9.2. Add enough **Nuclear stain** to cover the sample area.
- 9.3. Incubate for 2 to 10 seconds at room temperature. +++
- 9.4. Rinse the slides under running tap water (not deionized water).

10. Dehydration and mounting ^α

- 10.1. Wash slides in water for 5 min.
- 10.2. Rapid dehydrate slides with 2x 1 min wash in isopropanol.
- 10.3. Blot excess isopropanol from slides and apply VectaMount® Express Mounting Medium (H-5700-60).
- 10.4. Apply the coverslips and allow slides to dry flat at room temperature for 10 to 20 min.
- 10.5. Analyze using a brightfield microscope, using at least a 20x objective.
- 10.6. 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.

^α 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.