Item No. 39215

Naveni PD1/PD-L1 AP

Navinci

General guidelines

- Do not mix Naveni PD1/PD-L1 reagents with other Naveni ™ product lines.
- Reaction volume depends on the sample size.
- Use best practices when pipetting to minimize reagent consumption.
- Centrifuge vials before pipetting.
- Thoroughly defrost all buffer mixtures at room temperature, vortex well and spin down before use.
- Vortex and spin down all enzymes (1 and 2) before use.
- Keep enzymes on ice or a frozen cold block.
- Add enzymes right before adding reaction mix to sample.
- Remove excess washing buffer from samples before adding reagent.
- Do not allow slides/samples to dry.
- Preheat the humidity chamber before each step.
- Incubation times or temperatures other than those specified may compromise results.
- As with any product derived from biological sources, proper handling procedures should be used.
- Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.
- Chromogens may be carcinogenic and should be handled with care.
- Unused solutions should be disposed of according to local regulations.

Required but not supplied

- VectaMount® Express Mounting Medium (H-5700-60) from Vector Laboratories.
- Isopropanol 99,5 %.
- Endogenous alkaline phosphatase quenching solution.
- TBS and TBS-T – Tris-buffered saline and Tris-buffered saline supplemented with 0,05% Tween, respectively.

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

- Alkaline phosphatase
- 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. Wash any technical controls separately.

Kit components

Box 1.1:

Storage: +4 to +8°C. DO NOT FREEZE

Material	Art.no	Amount
NaveniBright Blocking Buffer (1x)	NB.1.100.01	4000 µl
NaveniBright Supplement 1	NB.1.100.03	500 µl
NaveniBright Antibody Diluent (1x)	NB.1.100.02	8000 µl
NaveniBright Supplement 2	NB.1.100.04	1000 µl
Probe Diluent (1x)	NF.1.100.03	4000 µl
NaveniBright Probe Anti-M (40x)	NB.1.100.06	100 µl
NaveniBright Probe Anti-R (40x)	NB.1.100.07	100 µl

Box 1.2:

Storage: +4 to +8°C. DO NOT FREEZE

Material	Art.no	Amount
NaveniBright AP/HRP diluent	NB.1.100.08	8000 µl
NaveniBright AP Reagent	NB.1.100.10	100 µl
NaveniBright AP Substrate Diluent	NB.1.100.09	8000 µl
NaveniBright AP Substrate 1	NB.1.100.11	140 µl
NaveniBright AP Substrate 2	NB.1.100.12	100 µl

Bag 1.3:

Storage: +4 to +8°C. DO NOT FREEZE

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

Box 2:

FREEZE

Storage: -25 to -15°C.

Material	Art.no	Amount
Navenibody PD1 (40x) based on clone EH33 CST	PPI.2.01	100 µl
Navenibody PDL1 (40x) based on clone SP142 Abcam RabMAb®	PPI.2.02	100 µl
NaveniBright Buffer 1 (5x)	NB.2.100.17	800 µl
NaveniBright Enzyme 1 (40x)	NF.2.100.11	100 µl
NaveniBright Buffer 2 (5x)	NB.2.100.18	800 µl
NaveniBright Enzyme 2 (40x)	NF.2.100.15	100 µl

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

Uppsala September 4 2022

Distributed by:
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Instructions of use

1. Sample preparation

1.1 After antigen retrieval, add enough alkaline phosphatase blocking solution (for quenching) (not provided) to cover each sample. Incubate for 10 min at room temperature, or according to manufacturer's user guide.
1.2 Wash slides for 2x5 min in 1x TBS-T**.

2. Blocking

2.1 Prepare blocking solution by adding 5 µl of Supplement 1 to every 40 µl of Blocking Buffer (1x).
2.2 Add the prepared blocking solution 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. Navenibody incubation

3.1 Prepare Navenibody solution by adding 5 µl of Supplement 2 to every 40 µl of Antibody Diluent (1x).
3.2 Use the prepared Navenibody solution to dilute Navenibody PD1 and Navenibody PDL1 to 1x (dilute 1:40 each).
3.3 Decant the blocking solution from slides and wash for 2x3 min in 1x TBS-T.
3.4 Add enough of the Navenibodies from step 3.2 to cover the sample area.
3.5 Incubate overnight at +4 °C in a humidity chamber.
3.6 Aspirate the antibody solution and wash slides for 3x5 min in 1x TBS-T in a staining jar under gentle agitation.

Kit component	Blocking solution	Navenibody solution
Blocking Buffer	40 µl	-
Supplement 1	5 µl	-
Antibody diluent	-	40 µl
Supplement 2	-	5 µl
Total:	45 µl	45 µl

4. Probe incubation

4.1 Prepare the probes by diluting Probe anti-M and Probe anti-R (dilute 1:40 each) in Probe Diluent (1x).
4.2 Add enough of the probes to cover the sample area.
4.3 Incubate for 60 min at +37 °C in a preheated humidity chamber.
4.4 Decant the solution and wash slides for 3x5 min in 1x TBS-T in a staining jar under gentle agitation.

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 (dilute 1:40) to the diluted buffer. Mix gently by pipetting, spin down and use immediatley.
5.3 Add enough 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 2x3 min in 1x TBS-T in a staining jar under gentle agitation.

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 (dilute 1:40) to the diluted buffer. Mix gently by pipetting, spin down and use immediatley.
6.3 Add enough 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 2x5 min in 1x TBS, followed by 1x10 min in 0,1x TBS in a staining jar under gentle agitation.
7.2 Dilute the NaveniBright AP reagent 1:300 in AP/ HRP diluent .
7.3 Decant wash buffer from the slides.
7.4 Add enough AP solution to cover the sample area.
7.5 Incubate for 30 min at room temperature with slow agitation.
7.6 Decant the solution and wash slides for 2x2 min in 1x TBS in a staining jar under gentle agitation.

8. Substrate development

8.1 Prepare the substrate solution by mixing AP Substrate Reagent 1 (dilute 62,5x) and AP Substrate Reagent 2 (dilute 80x) in A P Substrate Diluent . +

See calculation example for minimal volume:

Kit component	Substrate solution
AP Substrate Diluent	80 µl
AP Substrate 1	1,3 µl
AP Substrate 2	1,0 µl
Total:	82,3 µl

8.2 Decant wash buffer from the slides.
8.3 Add enough substrate solution to cover the sample area.
8.4 Incubate the slides at room temperature for 25 min. ++
8.5 Decant of the substrate solution from the slides and wash slides for 2x2 min in deionized water under gentle agitation.

9. Nuclei 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 ¤

10.1 Wash slides in water for 5 min with gentle agitation.
10.2 Rapid dehydrate slides with 2x1 min wash in isopropanol.
10.3 Blot excess isopropanol from slides and apply VectaMount® Express Mounting Medium (H-5700-60).
10.4 Apply coverslip 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. For fluorescence imaging, use a filter set for Texas Red.
10.6 After imaging, store the slides at room temperature. Signal is stable for years.

+	For alternative substrates, prepare according to manufacturer's 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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