



# SZABO SCANDIC

Part of Europa Biosite

## Produktinformation



Forschungsprodukte & Biochemikalien



Zellkultur & Verbrauchsmaterial



Diagnostik & molekulare Diagnostik



Laborgeräte & Service

Weitere Information auf den folgenden Seiten!  
See the following pages for more information!



### Lieferung & Zahlungsart

siehe unsere [Liefer- und Versandbedingungen](#)

### Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

### SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

[mail@szabo-scandic.com](mailto:mail@szabo-scandic.com)

[www.szabo-scandic.com](http://www.szabo-scandic.com)

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

## 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.



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.



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.



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

### Box 2:



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.

# 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 cm<sup>2</sup> 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 immediately.
- 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 immediately.
- 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 **AP 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 <sup>⊠</sup>

- 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.