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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

www.szabo-scandic.com

linkedin.com/company/szaboscandic

Naveni pY PD1, HRP KIT INSTRUCTIONS

Item No. 39212

GENERAL GUIDELINES:

- Do not mix NaveniBright 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.
- Wait to add enzymes until immediately before adding to the 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 give erroneous 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 horseradish peroxidase quenching solution.
- TBS and TBS-T – Tris-buffered saline and Tris-buffered saline supplemented with 0,05% Tween, respectively.

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:

Material	Article Number	Amount	Storage*
NaveniBright Blocking Buffer (1x)	NB.1.100.01	4000 µl	+4 to +8°C **DO NOT FREEZE**
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:

Material	Article Number	Amount	Storage*
NaveniBright HRP Reagent (800x)	NB.1.100.05	100 µl	+4 to +8°C **DO NOT FREEZE**
NaveniBright HRP Substrate 1	NB.1.100.13	170 µl	
NaveniBright HRP Substrate 2	NB.1.100.14	100 µl	
NaveniBright HRP Substrate 3	NB.1.100.15	100 µl	
NaveniBright HRP Substrate 4	NB.1.100.17	176 µl	

Bag 1.3:

Material	Article Number	Amount	Storage*
NaveniBright AP/HRP diluent	NB.1.100.08	8000 µl	+4 to +8°C **DO NOT FREEZE**
Nuclear Stain	NB.1.100.16	6000 µl	

Bag 2:

Material	Article Number	Amount	Storage*
Navenibody PD1 (200x)	PD1.2.01	20 µl	at -25 to -15°C
Navenibody pTyr M (200x)	PD1.2.02	20 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

1. Sample preparation

1.1 After antigen retrieval, add enough horseradish peroxidase blocking solution (for quenching) (not provided) to cover each sample. Incubate for 5 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^2 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 pTyr M** to 1x (dilute 1:200 each).
3.3 Decant the blocking solution from slides and wash for 2x3 min in 1x TBS-T.
3.4 Add enough of Navenibodies to cover the sample area.
3.5 Incubate overnight at +4 °C in a humidity chamber.
3.6 Decant 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	-
Primary 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 and spin down.
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 and spin down.
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. HRP 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 HRP reagent** 1:800 in **AP/ HRP diluent.**
7.3 Decant wash buffer from the slides.
7.4 Add enough HRP solution to cover the sample area.
7.5 Incubate for 30 min at room temperature with slow agitation.

8. Substrate development

8.1 Decant the solution and wash slides for 2x2 min in 1x TBS in a staining jar under gentle agitation.

8.2 Prepare the substrate solution by mixing **HRP Substrate 1** (dilute 62x), **HRP Substrate 2** (dilute 100x), **HRP Substrate 3** (dilute 100x) and **HRP Substrate 4** (dilute 62,5x) in distilled water. [+]
See calculation example for minimal volume:

Kit Component	Substrate Solution
Distilled water	100 µl
HRP Substrate 1	1,6 µl
HRP Substrate 2	1,0 µl
HRP Substrate 3	1,0 µl
HRP Substrate 4	1,6 µl
Total:	105,2 µl

8.3 Decant wash buffer from the slides.

8.4 Add enough substrate solution to cover the sample area.

8.5 Incubate the slides at room temperature for 5 to 10 min. [++]

8.6 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.

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.

Distributed by:
Cayman Chemical
1180 E. Ellsworth Rd.
Ann Arbor, MI 48101 USA

Customer Service: (800) 364-9897
Technical Support: (888) 526-5351
www.caymanchem.com