NaveniBright – MR, HRP KIT INSTRUCTIONS

Item No. 39213

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.

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:

Material	Article Number	Amount	Storage*
NaveniBright Blocking Buffer (1x)	NB.1.100.01	4000 µl	at +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	at +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	At +4 to +8°C **DO NOT FREEZE**
Nuclear Stain	NB.1.100.16	6000 µl	

Bag 2:

Material	Article Number	Amount	Storage*
NaveniBright Buffer 1 (5x)	NB.2.100.17	800 µl	at -25 to -15°C
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 quenching solution (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 supplementing 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 1cm^2 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 supplementing 5 µl of **Supplement 2** to every 40 µl of **Primary Antibody Diluent** (1x).

3.2 Use the prepared **primary antibody solution** to dilute your primary antibody or antibodies.

3.3 Decant the blocking solution from slides and wash for 2x3 min in 1x TBS-T.

3.4 Add enough of your 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 3x5 min with 1x TBS-T** in a staining jar under gentle agitation.

Kit component	Blocking Solution	Primary antibody 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** in **Probe Diluent** (1x) (dilute 1:40 each).

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 with 1x TBS-T in a staining jar under gentle agitation.

5. Reaction 1

5.1 Start preparing **Reaction 1** by diluting **Buffer 1** (5x) 1:5 in water. Vortex and spin down.
5.2 Add **Enzyme 1** (dilute 1:40). 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 with 1x TBS-T in a staining jar under gentle agitation.

6. Reaction 2

6.1 Start preparing **Reaction 2** by diluting **Buffer 2** (5x) 1:5 in water. Vortex and spin down.
6.2 Add **Enzyme 2** (dilute 1:40). Mix gently by pipetting and spin down.
6.3 Add enough Reaction 2 to cover the sample area.
6.4 Incubate for **60 min*** ** at +37 °C in a preheated humidity chamber.

7. HRP Incubation

7.1 Decant the solution and wash slides for 2x5 min with 1x TBS, followed by 1x10 min with 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 with 1x TBS in a staining jar under gentle agitation.
8.2 Prepare the substrate solution by mixing **HRP Substrate 1** (dilute 62,5x), **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 2 to 10 min. *****
8.6 Decant of the substrate solution from the slides and wash slides for 2x2 min in high purity water under gentle agitation.

9. Nuclei staining

9.1 Decant wash buffer from the slides.
9.2 Add enough nuclei stain to cover the sample area.
9.3 Incubate for 10 seconds at room temperature.
9.4 Rinse the slides under running tap 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 the mounting medium 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.

** TBS-T (Tris-buffered saline supplemented with 0,05% Tween 20).
*** Incubation time can be extended to 90 min for low abundance targets.
**** For alternative substrates, prepare according to manufacturer's user guide.
***** Substrate incubation time should be optimized for each assay.

¤ 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.