



NaveniBright – MR, HRP

KIT INSTRUCTIONS

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 1cm² 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 a

- 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.

a 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.