NC.MR.100 ??????????

NaveniBright - MR, HRP

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 PROVIDED

- 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:

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 HRP Reagent (800x)	NB.1.100.05	100 µl
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:

Storage: 4 to 8°C

DO NOT FREEZE

Material	Art.no	Amount
NaveniBright AP/HRP diluent	NB.1.100.08	8000 µl
Nuclear Stain	NB.1.100.16	6000 µl

Bag 2:

Storage: -25 to -15°C. Protect from light!

FREEZE

Material	Art.no	Amount
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 for at least 3 months after receipt.

For more information, or to place an order, visit
www.navinci.se/products
Email: contact@navinci.se

Uppsala August 21 2023

Instructions for use

1. Sample preparation

1.1. After antigen retrieval, add enough alkaline phosphatase/horseradish peroxidase 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**.

*TBS-T (Tris-buffered saline supplemented with 0.05% Tween 20).

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. Primary antibody incubation

3.1. Prepare primary antibody solution by adding 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 in 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 volume:	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 60 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 first substrate solution to cover the sample area (HRP).
8.5. Incubate the slides with substrate at room temperature for 2 to 20 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.

**	TBS-T (Tris-buffered saline supplemented with 0,05% Tween 20).
***	Incubation time can be extended to **90 min** for low abundance targets or intranuclear targets.
+	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.

Navinci

Guidelines for in situ PLA visualization of PD1 – SHP2 interaction using the NaveniBright kit

General guidelines

- The recommended PD1 and SHP2 antibodies have been optimized and validated to be used together and in combination with NaveniBright – MR, AP or NaveniBright – MR, HRP to enable visualization of the interaction between PD1 and SHP2 in formalin-fixed paraffin-embedded human tissues.
- For best result, follow the NaveniBright instructions included with your NaveniBright kit, applying the modifications described below.
- Note that your experiment set-up might need further optimization regarding concentrations and incubation time.
- For research use only. Not for use in diagnostic procedures.

Antibody	Vendor	Working centration	Storage
PD1 (D4W2J) Rabbit monoclonal antibody	Cell signalling technology	1 µg/ml	Store according to vendors instructions
SHP2 (6D9) Mouse monoclonal antibody	ThermoFisher	5 µg/ml	

1. Primary antibody incubation

Add the PD1 and SHP2 antibodies in step 3.2 of the NaveniBright protocol by diluting the antibodies to the recommended working concentration in primary antibody solution. For this antibody combination it is recommended to run the incubation in step 3.5 overnight.

2. Wash after probe incubation

Pre-heat the 1x TBS-T to 37°C and use the warm TBS-T to perform the 3x5 minutes washes in step 4.4 after the probe incubation.

3. Reaction 2

In step 6.4 of the NaveniBright protocol, allow the slides to incubate for 90 minutes.

4. Substrate development

In step 8.5 of the NaveniBright protocol, allow HRP substrate to develop for approximately 7 minutes and AP substrate to develop for approximately 25 minutes.

Navinci