Art.no 60054

Omni

Instructions For Use

For research use only

Manufactured by:

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Intended use

Omni enables the simultaneous detection of up to 9 protein-protein interactions, single protein expression and/or post translational modifications, with high specificity and sensitivity in FFPE human tissue and FFPE human cells.

Principles of the procedure

Omni is a multiplex *in situ* proximity ligation assay with a fluorescent readout. Using this assay, up to 9 separate targets, protein-protein interactions, single protein expression and/or post translational modifications can be detected within a single experimental workflow. For each target a unique pair of Navenibodies are used. The user can choose a unique panel from Navinci's library of available targets.

The detection of the targets is performed in up to 3 imaging cycles with each cycle detecting 3 targets and an optional 4th cycle to detect autofluorescence. The targets imaged within each cycle use separate fluorophores to make simultaneous detection possible.

Warnings and precautions

- For research use only. Not for use in diagnostic procedures.
- Not for internal or external use in humans or animals.
- As with any product derived from biological sources, proper handling procedures should be used.
- Wear appropriate personal protective equipment to avoid contact with reagents with eyes and skin.
- Always work in a fume hood when handling Xylene and hydrophobic pens.
- Exercise caution when handling hot solutions and equipment during and after the HIER procedure. Use appropriate heatresistant gloves and handle heated containers with care.

General guidelines

- Reaction volume depends on sample size and should be sufficient to cover the entire sample area. A volume of approximately 40 µL/cm^2 is recommended. For example, for an FFPE tissue section that covers an area of 5 cm^2 on a tissue section, we recommend you use a reaction volume of 200 µL/tissue section.
- Always prepare an excess volume to account for dead volume and pipetting losses from your workflow.
- Use best practices when pipetting to minimize reagent consumption.
- Thoroughly defrost all buffers at room temperature. Vortex well before use.
- Centrifuge vials before pipetting.
- Gently vortex and spin down Naveni Enzyme 1 (40x) and Naveni Enzyme 2 (40x) before use.
- Keep enzymes on ice or on a frozen cooling block.
- Dilute enzymes and mix well right before adding reaction mix to sample.
- Incubation times or assay temperatures other than those specified may negatively impact results.
- Warm 1x TBS-T is only required for washing in step 4.4, additional warm washing steps may lead to signal deterioration.
- Detection Mixes are light sensitive. Always keep them protected from light.
- Remove excess washing buffer from samples before adding the reagent mix.
- Do not allow slides/samples to dry.
- Preheat the humidity chamber before each step.
- Unused solutions should be disposed of according to local regulations.
- If image registration is performed as a pre-processing step, it is required to image the same regions of the sample for each cycle to ensure the greatest possible overlap.

Kit components

Omni Box 1:

Art. no. 60055
Storage: 2 to 8°C

DO NOT FREEZE

Material	Art.no	Amount
Naveni Block	50014	6 mL
Naveni Diluent	50001	8 mL
Omni Removal Buffer (201x)	50076	15 mL

Omni Box 2:

Art. no. 60056
Storage: -25 to -15°C

FREEZE

Material	Art.no	Amount
Naveni Enzyme 1 (40x)	50002	100 µL
Naveni Enzyme 2 (40x)	50003	100 µL
Omni Buffer 1 / A-I (5x)	50075	800 µL
Naveni Buffer 2 (5x)	50005	800 µL
Naveni Post Block (40x)	50006	100 µL
Omni Detection Mix / ABC (5x)	50072	800 µL
Omni Detection Mix / DEF (5x)	50073	800 µL
Omni Detection Mix / GHI (5x)	50074	800 µL

Omni Box 3:

Art. no. 60057

Box 3 includes oligo conjugated antibodies, Navenibodies. These are packed separately to make it possible for the user to customize the assay. By choosing different combinations of Navenibodies different panels can be created. To create your own panel with anything between 3 to 9 assays, visit our website: order.navinci.se

Each assay requires two Navenibodies provided in two vials with 40 µl each, and a 100x concentration, giving a final working volume of 4.0 ml. Nine oligonucleotide sets (A–I) are available for conjugation. Antibodies labeled with sets A-C are detected using Omni Detection Mix / ABC, sets D-F are detected using Omni Detection Mix / DEF and sets G-I are detected using Omni Detection Mix / GHI.

Required but not provided

Materials

- Pipettes and tips with appropriate volumes
- Tubes, beakers, and cylinders for reagent preparation
- Incubators 37°C and 60°C
- Pressure cooker
- Hydrophobic pen
- Vortex mixer
- Microcentrifuge
- Shaker
- Humidity chamber
- Staining jars
- Ice or a frozen cooling block for enzymes
- Coverslips
- Fluorescence microscope

Reagents

- Xylene
- Ethanol, 70, 95 and 99.5%
- Antigen Retrieval Buffer pH 9.0 (10x), Navinci, 60058
- Tris Buffered Saline with 0.05% Tween 20 (TBS-T)
- Tris Buffered Saline (TBS)
- Deionized water
- SlowFade™Gold Antifade Mountant, ThermoFisher Scientific, S36937*

**It is very important to use a mounting medium that is compatible with the assay as some available products will reduce or even remove the signal.*

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Instructions for use

1. Pre-treatment

The method for pre-treatment of tissue sections is described in the Instructions For Use of Antigen Retrieval Buffer pH 9.0 (10x), Navinci, 60058, IFU-0032.

2. Preparation of warm buffers

2.1 Prepare a sufficient volume* of 1x TBS-T and pre-warm to 37°C together with the required number of staining jars at least 12 h before use. Warm 1x TBS-T is required at step 4.4.
2.2 Prepare a sufficient volume* of Removal Buffer 1x working solution by diluting Omni Removal Buffer (201x) in water. (Example: 3 mL + 600 mL) and pre-heat to 60 °C in an incubator at least 12 h before use. Warm removal buffer working solution is required during the cycling at steps 11.3, 14.1 and 17.1.

**The volume needed is dependent on the volume and number of staining jars needed for the experiment. As an example, if you need two staining jars with a volume of 100 mL each, washing the slides 3 times in step 4.4, you will need 600 mL (2 x 100 mL x3) of pre-warmed buffer.*

3. Blocking

3.1 Add Naveni Block to the entire sample area.
3.2 Incubate for 60 min at 37 °C in a preheated humidity chamber.

4. Navenibody incubation

4.1 Dilute all Navenibodies for the selected panel 100x into one tube with Naveni Diluent. An example calculation for a total volume of 200 µL, sufficient for a sample area of ~5 cm^2, can be seen in table 2 below.

Table 2. Example calculation for a 200 µL Navenibody working solution.

Reagent for 5 cm^2 sample area	Volume
Naveni Diluent	164 µL
Target1.A1.XXXXX	2 µL
Target1.A2.XXXXX	2 µL
Target2.B1.XXXXX	2 µL
Target2.B2.XXXXX	2 µL
Target3.C1.XXXXX	2 µL
Target3.C2.XXXXX	2 µL
Target4.D1.XXXXX	2 µL
Target4.D2.XXXXX	2 µL
Target5.E1.XXXXX	2 µL
Target5.E2.XXXXX	2 µL
Target6.F1.XXXXX	2 µL
Target6.F2.XXXXX	2 µL
Target7.G1.XXXXX	2 µL
Target7.G2.XXXXX	2 µL
Target8.H1.XXXXX	2 µL
Target8.H2.XXXXX	2 µL
Target9.I1.XXXXX	2 µL
Target9.I2.XXXXX	2 µL
Total volume:	200 µL

4.2 Decant the Naveni Block and add a sufficient volume of the Navenibody working solution from step 4.1 to cover the sample area.
4.3 Incubate overnight at 4°C in a humidity chamber.
4.4 Decant the solution and wash the slides with 1x TBS-T pre-warmed to 37 °C from Step 2.1. Wash for 2x 10 sec and 1x 15min in the pre-warmed staining jar under gentle agitation. Wash controls separately.

5. Reaction 1

An example calculation for a total reaction volume of 200 µL, sufficient for a sample area of ~5 cm^2, can be seen in table 3.

Table 3. Example calculation for a 200 µL Reaction 1 working solution.

Reagent for 5 cm^2 sample area	Volume
H_2O	155 µL
Omni Buffer 1 / A-I (5x)	40 µL
Naveni Enzyme 1 (40x)	5 µL
Total volume:	200 µL

5.1 Start preparing Reaction 1 working solution by diluting Omni Buffer 1 / A-I (5x) in water. Vortex and spin down.
5.2 Add Naveni Enzyme 1 (40x). Mix gently by pipetting and spin down.
5.3 Dip the slides in 1x TBS for a few seconds and then remove excess liquid by gently tapping the slides on absorbent paper.
5.4 Add a sufficient volume of Reaction 1 working solution to cover the sample area.
5.5 Incubate for 30 min at 37 °C in a preheated humidity chamber.
5.6 Decant the solution, wash slides 1x 10 sec and 1x 5 min with 1x TBS-T in a staining jar under gentle agitation.

6. Reaction 2

An example calculation for a total reaction volume of 200 µL, sufficient for a sample area of ~5 cm^2, can be seen in table 4.

Table 4. Example calculation for a 200 µL Reaction 2 working solution.

Reagent for 5 cm^2 sample area	Volume
H_2O	155 µL
Naveni Buffer 2 (5x)	40 µL
Naveni Enzyme 2 (40x)	5 µL
Total volume:	200 µL

6.1 Start preparing Reaction 2 working solution by diluting Naveni Buffer 2 (5x) in water. Vortex and spin down.
6.2 Add Naveni Enzyme 2 (40x). Mix gently by pipetting and spin down.
6.3 Dip the slides in 1x TBS for a few seconds and then remove excess liquid by gently tapping the slides on absorbent paper.
6.4 Add a sufficient volume of Reaction 2 working solution to cover the sample area.
6.5 Incubate for 90 min at 37 °C in a preheated humidity chamber. Proceed to step 7.1 before the incubation is over.

7. Post block

7.1 While Reaction 2 is incubating, thaw and dilute Naveni Post block (40x) in Naveni Diluent (Example: 5 µL + 195 µL) Vortex and spin down.
7.2 After the incubation step is complete, decant Reaction 2 and add a sufficient volume of the Post block working solution from step 7.1 to cover the sample area.
7.3 Incubate for 30 min at 37 °C in a preheated humidity chamber. Proceed to step 8.1 before the incubation is over.

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CYCLE 1 / ABC
8. Detection / ABC (protect from light!)
8.1 Prepare the Detection Mix / ABC working solution by diluting Omni Detection Mix / ABC 5x in water (Example: 40 µL + 160 µL). Vortex and spin down.
8.2 Decant the Post block working solution. Do not wash but make sure the solution has been removed. Add a sufficient volume of Detection Mix / ABC working solution from step 8.1 to cover the sample area.
8.3 Incubate for 30 min at 37 °C in a preheated humidity chamber.
8.4 Decant the solution and wash slides 2x 5 min with 1x TBS in a staining jar under gentle agitation.
8.5 Perform a final 5 min wash in 0.1x TBS under gentle agitation.

Note: The three detection mixes all include DAPI. No extra step for DAPI staining is therefore necessary.

9. Mounting (protect from light!)
9.1 Dry slides in a slide centrifuge or air-dry them and mount them with a coverslip using SlowFade™Gold Antifade Mountant (not provided). After mounting, slides can be kept at +2 to +8 °C for up to 4 days before imaging.

10. Imaging
10.1 Image your slides on a fluorescence or confocal microscope using a 20x objective or higher.
10.2 For imaging, a filter set corresponding to your chosen nuclear stain and the detection fluorophore's excitation/emission spectra is required, see table 5.

Table 5. Filter sets for imaging.

Filter set	Detecting	λ Excitation	λ Emission
DAPI	Nuclei	360-370 nm	450-460 nm
Cy5	Proximity signal detected with sets A, D, G	635-645 nm	665-675 nm
Cy3	Proximity signal detected with sets B, E, H	545-555 nm	575-585 nm
FITC	Proximity signal detected with sets C, F, I	480-490 nm	525-535 nm

CYCLE 2 / DEF
11. Removal of coverslip and detection oligos
11.1 Place the mounted slides in 1x TBS-T at room temperature for 15 minutes under gentle agitation to remove coverslips.*
11.2 After the coverslips have fallen off, wash the slides 2 min with 1x TBS-T in a staining jar under gentle agitation.
11.3 Place the slides in Omni Removal Buffer 1x working solution pre-heated to 60 °C and put them in a 60 °C incubator 2x 10 minutes. Change the pre-heated Omni Removal Buffer 1x working solution between the two incubations.
11.4 Wash the slides for 2 min with 1x TBS in a staining jar.

** Coverslips should fall off by themselves. Avoid trying to manually remove them by applying any force. This could damage the sample. If using a hardening mounting medium (Fluoroshield™ Sigma-Aldrich F6182), use 1x TBS-T pre-heated to 37 °C and put them in a 37 °C incubator for 20-120 minutes. The time it takes for the coverslips to fall off when using a hardening mounting media varies and depends on the time the coverslip has been mounted. If 24 hours or more has passed from the mounting of the coverslip, an overnight removal in 1x TBS-T in a staining jar at room temperature under gentle agitation is recommended.*

12. Detection / DEF (protect from light!)
12.1 Prepare the Detection Mix / DEF working solution by diluting Omni Detection Mix / DEF 5x in water (Example: 40 µL + 160 µL). Vortex and spin down.
12.2 Decant the TBS from the slides. Add a sufficient volume of Detection Mix / DEF working solution from step 12.1 to cover the sample area.
12.3 Incubate and wash according to 8.3 to 8.5 above.

13. Mounting and Imaging (protect from light!)
13.1 Perform mounting and imaging according to sections 9. and 10. above.

CYCLE 3 / GHI
14. Removal of coverslip and detection oligos
14.1 Perform removal of coverslip and detection oligos according to section 11. above.

15. Detection / GHI (protect from light!)
15.1 Prepare the Detection Mix / GHI working solution by diluting Omni Detection Mix / GHI 5x in water (Example: 40 µL + 160 µL). Vortex and spin down.
15.2 Decant the TBS from the slides. Add a sufficient volume of Detection Mix / GHI working solution from step 15.1 to cover the sample area.
15.3 Incubate and wash according to sections 8.3 to 8.5 above.

16. Mounting and Imaging (protect from light!)
16.1 Perform mounting and imaging according to sections 9. and 10. above.

CYCLE 4 / Autofluorescence
17. Removal of coverslip and detection oligos
17.1 Perform removal of coverslip and detection oligos according to section 11. above.

18. Mounting and Imaging
18.1 Perform mounting and imaging according to sections 9. and 10. above.

Image Registration Guidelines
Image registration is the process of aligning images acquired from the different staining cycles. Registration also facilitates background removal by subtracting the autofluorescence channels acquired in cycle 4. A guideline for image registration can be found on the Navinci website. It is recommended to read through the requirements in the image registration guideline before running the experiments.

www.navinci.se/resources/image-registration-guidelines

QuPath Image Analysis Guidelines
Image analysis is a critical step for interpretation of multiplex data generated by Navinci *in situ* proximity ligation assays (*is*PLA). A guideline for image analysis using QuPath is available on the Navinci website. The guideline includes instructions on how to use QuPath, how to inspect and explore images using the image viewer, and how to perform digital image analysis steps such as signal detection and cell segmentation.

www.navinci.se/resources/qupath-guidelines

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