

Art. No. 60060

NaveniBright™ AUTO

Art. No. 60061

NaveniBright™ PD1/PD-L1 AUTO

Instructions For Use

For research use only

Manufactured by:

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Intended use

NaveniBright™ AUTO

The NaveniBright™ AUTO (Art. No. 60060) enables automation of Navinci's *in situ* proximity ligation assay (isPLA) technology on open automated staining platforms. Using two unlabeled primary antibodies, the assay can be used to study protein-protein interaction through molecular proximity, protein expression, and protein post-translational modifications in FFPE samples.

NaveniBright™ PD1/PD-L1 AUTO

NaveniBright™ PD1/PD-L1 AUTO (Art. No. 60061) enables automation of an *in situ* proximity ligation assay (isPLA) for detection of the interaction between PD1 and PD-L1 in FFPE samples on compatible automated staining platforms. Primary antibodies are included in the kit.

Principles of the procedure

These products are *in situ* proximity ligation assays. This means that they detect the close proximity binding of two separate antibodies. By using antibodies directed against two separate proteins a protein-protein interactions can be studied. If instead using antibodies binding two epitopes on the same protein specific single protein expression is detected. It is also possible to study post translational modification if using one antibody directed against a protein and a second antibody directed against a modification of the same protein.

The detection requires two unlabelled primary antibodies, one each from mouse and rabbit hosts. With NaveniBright™ AUTO, primary antibodies are selected by the user, providing flexibility to develop and evaluate different assays using the same kit. With NaveniBright™ PD1/PD-L1 AUTO, primary antibodies for detection of the interaction between PD1 and PD-L1 are included in the kit.

The NaveniBright™ products are HRP-based assays. The assay generates localized HRP labeling that requires a compatible HRP detection system for signal visualization. Detection reagents are **not** included in the kit and must be supplied by the user.

Implementation requires an open automated staining platform that allows user-defined protocol development and use of user-supplied reagents.

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.
- Follow all safety instructions provided by the manufacturer of the automated staining platform and any third-party reagents used in the workflow.

Kit components

Box 1

Art. No. 60062

Storage: +2 to +8°C

DO NOT
FREEZE



Name	Art.no.	Amount
NaveniBright Block	50077	7.2 mL
Naveni Supplement 1	50078	900 µL
Naveni Supplement 2	50079	1.8 mL
Naveni Diluent	50015	2x 15 mL
Navenibody M1	50050	300 µL
Navenibody R2	50052	300 µL
Naveni HRP Diluent	50016	8.0 mL
Naveni HRP Reagent	50080	35 µL

Box 2

Art. No. 60063

Storage: -25 to -15°C

FREEZE



Name	Art.no.	Amount
Naveni Buffer 1 (5x)	50081	1.7 mL
Naveni Enzyme 1 (20x)	50035	2x 300 µL
NaveniBright Buffer 2 (5x)	50082	2.7 mL
Naveni Enzyme 2 (20x)	50036	2x 530 µL

Bag 1

Art. No. 60064

Storage: +2 to +8°C

DO NOT
FREEZE



Name	Art.no.	Amount
Naveni PD1 Antibody	50085	200 µL
Naveni PD-L1 Antibody	50086	200 µL

Note: Bag 1 is only included in the NaveniBright™ PD1/PD-L1 AUTO, art.no. 60061

General guidelines

- Reaction volume requirements depend on the automation platform used. Follow platform-specific protocols when available or optimize the volume for your own system.
- 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.
- A precipitate may be present in Naveni Buffer 1 during storage. This is expected and will dissolve upon thawing and mixing.
- Keep the enzyme stocks on ice or in a frozen cold block during preparation.
- Gently vortex and spin down Naveni Enzyme 1 (20x) and Naveni Enzyme 2 (20x) before use.
- After preparation of each working solution, mix thoroughly to ensure a homogeneous reagent mixture. Mixing by pipetting is recommended. Avoid introducing bubbles.
- All NaveniBright™ AUTO reagents are bench-top stable for at least 24 hours after preparation when stored at room temperature.
- Unused solutions should be disposed of according to local regulations.
- NaveniBright™ AUTO is an HRP-based assay. Signal visualization requires a compatible HRP detection system supplied by the user.
- Use a mounting medium that is compatible with the selected HRP detection system and detection chemistry.

Required but not provided

Materials and reagents required are specific to the automation platform used to run the assay. Information about platform-specific materials, reagents and consumables is provided in the applicable Navinci Technical Notes.

Materials

- Pipettes and tips with appropriate volumes
- Tubes or containers suitable for reagent preparation
- Vortex mixer
- Microcentrifuge
- Ice or a frozen cooling block for enzymes
- Coverslips
- Microscope or imaging system compatible with the selected detection chemistry
- Compatible automated staining platform

Reagents

- Mouse primary antibody and rabbit primary antibody (NaveniBright™ AUTO only)
- Antigen retrieval reagents compatible with the selected workflow (e.g. Antigen Retrieval Buffer pH 9.0 (10x), Navinci, Art. No. 60058)
- Wash buffers compatible with the selected workflow
- Deionized water
- HRP detection reagents compatible with the selected workflow (e.g. DAB, TSA- or Opal-based detection systems) *
- Instrument-specific reagents and consumables required by the selected automation platform
- Counterstain (optional)
- Mounting medium compatible with the selected detection chemistry**

* NaveniBright™ AUTO is an HRP-based assay. Signal visualization requires a compatible HRP detection system ordered separately. Both chromogenic and fluorescence-based detection systems may be used.

** Selection of mounting medium may affect signal quality. Use a mounting medium that is compatible with the selected detection chemistry and workflow. Incompatible mounting media may reduce signal intensity or negatively affect sample quality.

Instructions for use

Instrument specific protocols

NaveniBright™ AUTO and NaveniBright™ PD1/PD-L1 AUTO are intended for use on open automated staining platforms that support user-defined protocols and user-supplied reagents.

Platform-specific Technical Notes are available for selected instruments. Please contact Navinci for information about available protocols.

For platforms not covered by a Navinci Technical Note, a general workflow is provided below, and assay conditions may need to be established by the user.

Recommended starting conditions

The workflow below can be used as a starting point when implementing NaveniBright™ AUTO on an automated staining platform for which no Navinci Technical Note is available. Optimization of reagent volumes, incubation times, wash conditions and detection chemistry may be required depending on the platform and assay. When adapting the workflow to a new platform, ensure that reagent volumes, incubation conditions and instrument settings remain within the operating specifications of the automated staining platform.

Step	Typical conditions	Notes
Sample preparation	According to sample and instrument requirements	Includes tissue preparation, baking of slides, deparaffinization and antigen retrieval.
Blocking	60 minutes at 37°C	Recommended starting condition.
Primary antibody incubation	60 minutes at 37°C	Optimize according to target and antibody pair.
Wash	≥ 3 washes	Recommended starting condition.
Navenibody incubation	60 minutes at 37°C	Sensitive assay step. May require optimization of incubation time, reagent concentration and use of multiple dispenses.
Wash	Thorough washing recommended (e.g 4x 10 minutes) at 37°C	Critical washing step. Thorough washing is recommended and should be considered for optimization if unwanted background staining is observed.
Reaction 1	30 minutes at 37°C	Recommended starting condition.
Wash	≥ 3 washes	Recommended starting condition.
Reaction 2	90 minutes at 37°C	Sensitive assay step. May require optimization of incubation time.
Wash	≥ 3 washes	Recommended starting condition.
HRP labeling	30 minutes at 37°C	Recommended starting condition.
Wash	≥ 3 washes	Recommended starting condition.
Signal detection	According to detection system	Use a compatible HRP detection system
Counterstaining (optional)	According to manufacturer's instructions	
Mounting	According to manufacturer's instructions	Use a mounting medium compatible with the selected detection chemistry.
Imaging	According to detection system	Use a compatible microscope or imaging platform

The workflow above is intended as a starting point for implementation of NaveniBright™ AUTO on automation platforms for which no Navinci Technical Note is available.

The primary antibody incubation, Navenibody incubation and Reaction 2 steps may require optimization depending on the selected target, antibody pair, sample type and automation platform.

Unless otherwise specified by the selected workflow or detection system, TBS-T is recommended as wash buffer between assay steps.

General reagent preparation

The reagent preparation instructions below may be used as a starting point when implementing NaveniBright™ AUTO on automation platforms for which no Navinci Technical Note is available. Prepare reagent volumes according to the requirements of the selected workflow and instrument.

Reagent	Preparation
Blocking solution	Dilute Naveni Supplement 1 1:9 in NaveniBright Block.
Antibody diluent	Dilute Naveni Supplement 2 1:9 in Naveni Diluent. Use the prepared solution to dilute primary antibodies.
Primary antibody solution	Dilute mouse and rabbit primary antibodies in Antibody Diluent according to the antibody manufacturer's recommendations or assay optimization. For NaveniBright™ PD1/PD-L1 AUTO, dilute the supplied PD1 and PD-L1 antibodies 1:40 in Antibody Diluent.
Navenibody solution	Dilute Navenibody M1 and Navenibody R2 1:40 each in Naveni Diluent.
Reaction 1	Dilute Naveni Buffer 1 (5x) 1:5 and Naveni Enzyme 1 (20x) 1:20 in deionized water. Add Naveni Enzyme 1 last and mix thoroughly.
Reaction 2	Dilute NaveniBright Buffer 2 (5x) 1:5 and Naveni Enzyme 2 (20x) 1:20 in deionized water. Add Naveni Enzyme 2 last and mix thoroughly.
HRP labeling solution	Dilute Naveni HRP Reagent 1:200 in Naveni HRP Diluent.