Navinci Universal 25 KIT INSTRUCTIONS

Catalog number ND0200 **Specificity:** Mouse&Rabbit

1. Blocking	1.1	Add **Blocking Buffer** (1x) to the entire sample area (approximately 40 µl for each 1cm^2 area).
	1.2	Incubate for 60 min at +37 °C in a pre-heated humidity chamber.
2. Primary antibody incubation	2.1	Use the provided **Primary Antibody Diluent** (1x) to dilute your primary antibodies.
	2.2	Decant the Blocking Buffer and add enough of your antibodies to cover the sample area.
	2.3	Incubate for 60 min at +37 °C or overnight at +4 °C in a humidity chamber.
	2.4	Decant the antibody solution and wash slides for 3x5 min with 1x TBS-T* in a staining jar under gentle agitation.
3. Probe incubation	3.1	Prepare the probes by diluting **Probe M1** and **Probe R2** in Probe Diluent (dilute 1:40 each).
	3.2	Add enough of the probes to cover the sample area.
	3.3	Incubate for 60 min at +37 °C in a pre-heated humidity chamber.
	3.4	Decant the solution and wash slides for 3x5 min with 1x TBS-T in a staining jar under gentle agitation.
4. Reaction A	4.1	Start preparing Reaction A by diluting **Buffer A** (5x) 1:5 in water. Vortex and spin down.
	4.2	Add **Enzyme A** (dilute 1:40) Mix gently by pipetting and spin down.
	4.3	Add enough Reaction A to cover the sample area.
	4.4	Incubate for 60 min at +37 °C in a pre-heated humidity chamber.
	4.5	Decant the solution and wash slides for 2x3 min with 1x TBS-T in a staining jar under gentle agitation.
5. Reaction B	5.1	Start preparing Reaction B by diluting **Buffer B** (5x) 1:5 in water. Vortex and spin down.
	5.2	Add **Enzyme B** (dilute 1:40). Mix gently by pipetting and spin down.
	5.3	Add enough Reaction B to cover the sample area.
	5.4	Incubate for 30 min at 37 °C in a pre-heated humidity chamber.
	5.5	Wash slides for 2x3 min with 1x TBS-T in a staining jar under gentle agitation.
6. Reaction C	6.1	Start preparing Reaction C by diluting **Buffer C** (5x) 1:5 in water. Vortex and spin down.
Protect from light	6.2	Add **Enzyme C** (dilute 1:40). Mix gently by pipetting and spin down.
	6.3	Add enough Reaction C to cover the sample area.
	6.4	Incubate for 90 min at +37°C in a pre-heated humidity chamber.
	6.5	Decant the solution and wash slides for 2x10 min with 1x TBS in a staining jar under gentle agitation.
	6.6	Wash slides for 15 min with 0.1x TBS in a staining jar under gentle agitation.
7. Mounting	7.1	Tap off excess wash buffer from the slides.
Protect from light	7.2	Mount the slides with a coverslip using water-based anti-fade mounting medium with DAPI.
	7.3	Wait for 15 minutes.
	7.4	Image your slides in a fluorescence or confocal microscope, using 20x objective or higher and filter set for DAPI and Texas red.

KIT COMPONENTS

Box 1: Catalog number ND0210

Material	Product Number	Amount	Storage
Blocking Buffer (1x)	ND00.1.1s	1000 µl	2–8°C DO NOT FREEZE
Primary Antibody Diluent (1x)	ND00.1.7s	1000 µl	
Probe Diluent (1x)	ND00.1.2s	1000 µl	
Probe M1 (40x)	ND00.1.4s	25 µl	
Probe R2 (40x)	ND00.1.3s	25 µl	

Box 2: Catalog number ND0220

Material	Product Number	Amount	Storage
Buffer A (5x)	ND00.2.1s	200 µl	-15 – -20°C PROTECT FROM LIGHT
Enzyme A1 (40x)	ND00.2.2s	25 µl	
Buffer B (5x)	ND00.2.3s	200 µl	
Enzyme B (40x)	ND00.2.4s	25 µl	
Buffer C (5x)	ND00.2.5s	200 µl	
Enzyme C (40x)	ND00.2.6s	25 µl	

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

General guidelines

- Reaction volume depends on the sample size.
- Use best practices when pipetting to minimize reagents consumption.
- Centrifuge vials before pipetting.
- Completely defrost all buffer mixtures (A, B, C) at room temperature, vortex well before use.
- Vortex and spin-down all enzymes (A, B and C) before use.
- Keep enzymes on ice or on a frozen cold block.
- Wait to add enzymes until immediately prior to adding to the sample.
- Buffer C is a light-sensitive reagent. Always keep it protected from light.
- 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.
- Unused solution should be disposed according to local regulation.

IMPORTANT: Appropriate precautions should be taken to avoid antibodies cross-contamination. Cross-contamination is the main source of unspecific background due to the high sensitivity of the assay.
Avoid bulk washing methods when multiple antibodies are used.