GENERAL GUIDELINES:

- Reactions volume depends on the sample size.
- Use best practices when pipetting to minimize reagents consumption.
- Centrifuge vials before pipetting.
- The final working concentration of different detergents for permeabilization (ex. Tween-20, Triton X100, digitonin) should be explored to find the optimal conditions to best preserve the cell structure and protein of interest.
- Select the Buffer C vial with the appropriate detection fluorophore for your microscope filter set.
- Available fluorophores are Texas Red, Atto 647, and Atto 488. The standard kit is equipped with Texas Red; if other fluorophore is needed, contact Navinci Diagnostics at contact@navinci.se
- Thoroughly defrost all buffer mixtures at room temperature, vortex well before use.
- Vortex and spin down all enzymes (A, B, and C) before use.
- Keep enzymes on ice or a frozen cold block.
- Wait to add enzymes until immediately before 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.
- Naveni assays might be combined with traditional immunofluorescence techniques, providing that all primary antibodies are from different species and the fluorescence detection systems use different fluorophores.
- 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 solutions should be disposed of according to local regulations.

IMPORTANT: Cross-contamination is the main source of unspecific background due to the high sensitivity of the assay.

Naveni pTyr EGFR KIT INSTRUCTIONS

Item No. 39206

KIT COMPONENTS:

Box 1:

Material	Article Number	Amount	Storage*
Blocking Buffer (1x)	NF.1.100.01	4000 µl	at +4 to +8°C DO NOT FREEZE!!!
Navenibody Diluent (1x)	NPT.1.100.01	4000 µl	

Box 2:

Material	Article Number	Amount	Storage*
EGFR Navenibody (40x)	NPT.2.17	100 µl	at -25 to -15°C
pTyr R Navenibody (40x)	NPT.2.21	100 µl	
Buffer A (5x)	NF.2.100.08	800 µl	
Enzyme A (40x)	NF.2.100.09	100 µl	
Buffer B (5x)	NF.2.100.10	800 µl	
Enzyme B (40x)	NF.2.100.11	100 µl	
Buffer C (5x), Texas Red	NF.2.100.12	800 µl	
Enzyme C (40x)	NF.2.100.15	100 µl	

1.Permeabilization (not provided)	1.1	Only for fresh frozen cell slides: Permeabilize cells with 0,05% Triton X-100 in PBS for 5 min at room temperature.
	1.2	Wash slides for 2x2 min with 1x PBS.
2. Blocking	2.1	Add **Blocking Buffer** (1x) to the entire sample area (approximately 40 µl for each 1cm^2 area).
	2.2	Incubate for 30 min at +37 °C in a pre-heated humidity chamber.
3. Navenibody incubation	3.1	Prepare Navenibodies by diluting **EGFR Navenibody (40x)** and **pTyr R Navenibody (40x)** in **Navenibody Diluent** (1x) (dilute 1:40 each).
	3.2	Add enough of the Navenibodies 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	Select the **Buffer C** vial with the appropriate detection fluorophore for your microscope filter set. Do not use more than one **Buffer C** vial.
Protect from light	6.2	Start preparing **Reaction C** by diluting **Buffer C** (5x) 1:5 in water. Vortex and spin down.
	6.3	Add **Enzyme C** (dilute 1:40). Mix gently by pipetting and spin down.
	6.4	Add enough Reaction C to cover the sample area.
	6.5	Incubate for 90 min at +37 °C in a pre-heated humidity chamber.
	6.6	Decant the solution and wash slides for 2 min with 1x TBS in a staining jar under gentle agitation.

7. Nuclei staining (not provided)	7.1	Start preparing a Nuclei staining solution according to the manufacturer's instruction. Vortex and spin down.
	7.2	Decant wash buffer from the slides.
	7.3	Add enough Nuclei staining solution to cover the sample area.
Protect from light	7.4	Incubate according to the manufacturer's instruction.
	7.5	Decant the solution and wash slides for 2x 10 min with 1x TBS in a staining jar under gentle agitation.
	7.6	Wash slides for 15 min with 0.1x TBS in a staining jar under gentle agitation.
8. Mounting (not provided)	8.1	Decant excess wash buffer from the slides.
	8.2	Mount the slides with a coverslip using a Fluoroshield anti-fade mounting medium.
Protect from light	8.3	Image your slides in fluorescence or confocal microscope, using 20x objective or higher and filter set for DAPI and a corresponding fluorophore (FITC for Atto 488, Texas Red, or Cy5 for Atto 647, respectively).

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