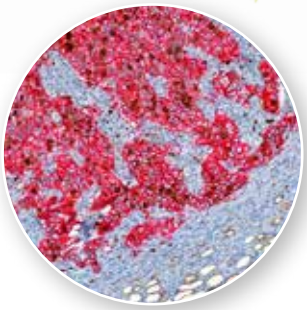
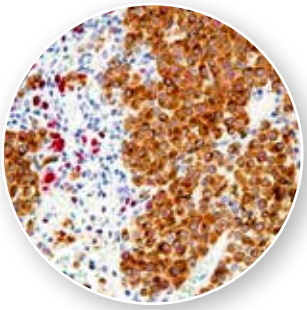
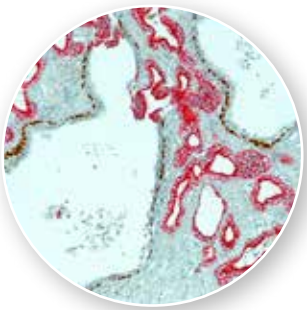


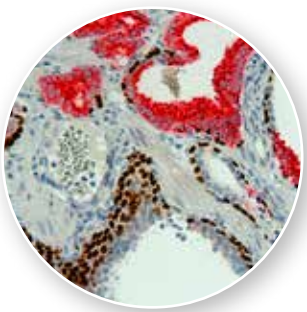
Ki-67 (RBK027) + HER2 (MSK044)
staining on mamma carcinoma



MART1 (MSK056) + Ki-67 (RBK027)
staining on malignant melanoma



PIN Cocktail (p63 + P504S; C0001K)
staining on prostate carcinoma



PIN Cocktail (p63 + P504S; C0001K)
staining on prostate carcinoma

Sequential double stain protocol for immunohistochemistry

Immunohistochemical double stains are easily performed by using Zytomed Systems Double Stain Polymer Detection Kit which uses a 1-step detection technique. The sequential protocol described here is a 2-step technique and, thus, slightly longer but also very easy. One primary antibody must be from mouse, the other one from rabbit.

Some remarks for this protocol:

- Both primary antibodies should need the same pre-treatment.
- Both primary antibodies should have different target cell structures (i.e. one primary antibody stains the cytoplasm, the other one the nucleus).
- The primary antibodies can be applied in a mixture on the slide or in sequential steps.
- One antibody has to be from mouse, the other one from rabbit.
- Both chromogenic substrates should be suitable for permanent mounting.

Protocol for IHC double staining:

- Deparaffinise and rehydrate paraffin-embedded tissue sections
- Apply and incubate Peroxide Block (3 % H_2O_2 solution) for 10 min
- Rinse with tap water
- Pre-treatment with HIER (Heat Induced Epitope Retrieval) or enzymatic digestion
- Rinse with Wash Buffer
- Apply and incubate the primary antibodies for 60 min; either in a combined incubation or one after another.
- Apply and incubate HRP Polymer anti-Mouse for 30 min
- Rinse with Wash Buffer
- Apply and incubate DAB working solution for 10 min
- Rinse with Wash Buffer
- Apply and incubate AP-Polymer anti-Rabbit for 30 min
- Rinse with Wash Buffer
- Apply and incubate Permanent AP-Red working solution for 20 min
- Rinse with tap water
- Counterstain with haematoxylin
- Blueing with tap water
- Dehydrate through a graded series of ethanol and clear in xylene
- Mount with a permanent mounting medium

Reagents used:

Peroxide Block	ZUC019-100
HIER Citrate Buffer pH 6.0	ZUC028-500
Wash Buffer	ZUC020-500
ZytoChem Plus HRP Polymer anti-Mouse	ZUC050-006
DAB Substrate Kit	DAB530
ZytoChem Plus AP Polymer anti-Rabbit	ZUC031-006
Permanent AP Red	ZUC001-125