

## 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:

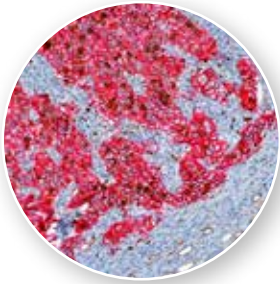
- ▶ Both primary antibodies should need the same pre-treatment.
- ▶ Both primary antibodies should have different target cell structures (i.e. one primary antibody stains the cytoplasm, the other one the nucleus).
- ▶ The primary antibodies can be applied in a mixture on the slide or in sequential steps.
- ▶ 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:

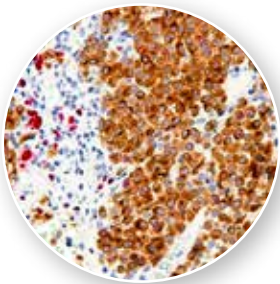
- ▶ Deparaffinise and rehydrate paraffin-embedded tissue sections
- ▶ Apply and incubate Peroxide Block (3% H<sub>2</sub>O<sub>2</sub> solution) for 10 min
- ▶ Rinse with tap water
- ▶ Pre-treatment with HIER (Heat Induced Epitope Retrieval) or enzymatic digestion
- ▶ Rinse with Wash Buffer
- ▶ Apply 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:

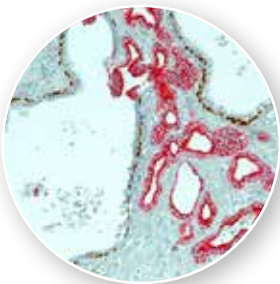
Peroxide Block .....	ZUC019-100
HIER Citrate Buffer pH 6.0 .....	ZUC028-500
Wash Buffer .....	ZUC020-500
ZytoChem Plus HRP Polymer anti-Mouse .....	ZUC050-006
DAB Substrate Kit .....	DAB530
ZytoChem Plus AP Polymer anti-Rabbit .....	ZUC031-006
Permanent AP Red .....	ZUC001-125



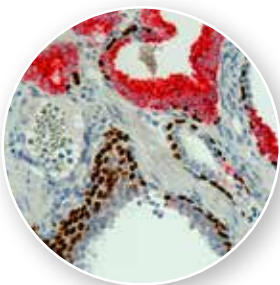
Ki-67 (RBK027) + HER2 (MSK044)  
staining on mamma carcinoma



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



PIN Cocktail (p63 + P504S; CO001K)  
staining on prostate carcinoma



PIN Cocktail (p63 + P504S; CO001K)  
staining on prostate carcinoma