

Immunohistochemistry using Polyclonal Antibody to CD44var(v3-v10) (human) (Prod. No. ALX-210-234):

Study protocol for testing the immunoreactivity on paraffin sections using wet autoclave and microwave pretreatment for antigen retrieval.

Patients material:

Routinely formalin fixed and paraffin embedded biopsy material of 3 squamous cell carcinomas of the oral cavity, 3 breast carcinomas and colonic adenocarcinomas retrieved from the files of the Institute of Pathology, University of Münster

Handling of the sections:

1-2µm thick paraffin sections were cut and mounted on poly-L-lysine coated glass slides. In cases of breast cancer different "glue" techniques were tested because of the frequent loss of sections during pretreatment procedures: the best results were achieved using either concentrated poly-L-lysine coated slides (without dilution layered over the precleaned glass slides) or by using Super Frost or Super Frost Plus slides (Menzel, Germany).

Dewaxing of the sections (as for routine immunohistochemistry):

2x15 minutes in xylene, 3 minutes in 100% ethanol, 3 minutes in 90% ethanol, 3 minutes in 70% ethanol, 5 minutes in distilled water.

Antigen retrieval using the wet autoclave protocol (see Bankfalvi et al. J. Pathol. 1994. 174, 223):

Dewaxed sections were immersed in sodium citrate buffer (0.01M sodium citrate monohydrate, pH 6.0) in plastic Coplin jars and incubated in a Goessner Laborautoklav GLA 40-2 for 5 minutes.

The procedures listed above are intended only as a guide. Various assay conditions require that the investigator determine the optimal working concentrations. The results may vary depending on experimental conditions and technique. No warranty or guarantee of performance of above procedure is made or implied. Use good laboratory practices and handle all materials with care.

These products and procedures are for in vitro experimental use only and are not intended for use in humans or clinical diagnosis.