

## **Western Blot using MAb to CD44var(v3) (human) (VFF-327v3) (Prod. No. ALX-801-079):**

### **1. SDS electrophoresis**

### **2. Transfer (use Immobilon PVDF or Hybond nitro-cellulose (Amersham))**

- Activate in methanol for 1-2 min.
- Wash with water for 5 min.
- Incubate in transfer buffer for 5 min. (15,1 g TRIS, 72,1g glycine, 1'000 ml methanol, water up to final 5'000 ml, pH 8,5).
- Bring gel/membrane/3MM paper together.
- Transfer over night (330 mA for 28 x 18 cm gels) or equivalent.

### **3. Immunoblot**

- Block with PBS/10% non-fat dry milk/0,3% Tween 20 over night at 4°C or 1 hour at 37°C.
- Wash two times with PBS/0,3% Tween 20 for 5 min.
- MAb to CD44var(v10) (human) (VFF-14): 10µg/ml in PBS/5% non-fat dry milk/0,3% Tween 20.  
→ If you do not see anything at this concentration there is not enough detectable protein; under normal conditions 1µg/ml is sufficient.
- Incubate 1 hour at room temperature.
- Wash 4 times with PBS/0,3% Tween 20 for 5 min.
- Second antibody: rabbit anti-mouse-HRPO 1:1'000 in PBS/5% non-fat dry milk/0,3% Tween 20 for 1 hour at room temperature.
- Wash 4 times with PBS/0,3% Tween 20 for 5 min.

### **4. Detection with ECL system from Amersham:**

- Mix solution 1 and solution 2 in equal amounts.  
Incubate blot for 1 min. at room temperature in this mixture.
- Dry blot carefully with tissue.
- Cover blot in Saran Wrap.  
Lay "side of gel" to X-ray film.  
Expose for 5 sec to 10 min.

## **Study protocol for testing the immunoreactivity of MAb to CD44var(v3) (human) (VFF-327v3) (Prod. No. ALX-801-079):**

**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:** 1-2 µm 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: 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):** 2 x 15 min. in xylene, 3 min. in 100% ethanol, 3 min. in 90% ethanol, 3 min. in 70% ethanol, 5 min. in distilled water

**Antigen retrieval** using the wet autoclave protocol (Bankfalvi et al. J. Pathol. 1994. 174:223-228).

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

**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.**