

Western Blot using Monoclonal Antibody to CD44var(v10) (human) (VFF-14) (Prod. No. ALX-801-084):

Western Blot Protocol:

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.

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.