## Properties

**Name:**

Western blotting -- immunoblot

**For what:**

Yeast protein analysis by immunoblotting

**Materials:**

Membrane: nitrocellulose

**Procedure:**

1. Blocking: leave the membrane in a box with a solution of PBS-T and 5% milk (30 ml) or 5% BSA on an oscillating table (the choice between milk or BSA depend on the primary antibody). This can last 1 hour at room temperature or days at 4°C. 2. Wash briefly with PBS-T to remove excess of milk or BSA. 3. Incubate with primary antibody in a 10ml solution in a falcon tube. For the antibody solution, see protocols in section "WESTERN\_BLOTTING" of openBIS. Incubate on the wheel for 1 hour at room temperature or let it overnight at 4°C on a wheel. 4. Wash the membrane: 4 X 5 min in PBS-T on the oscillating table. 5. Incubate with secondary antibody for 1 hour on the oscillating table. You can use a box with 30 ml PBS-T and 1% milk solution plus properly diluted secondary antibody. 6. Wash the membrane 2 X 5 min in PBS-T then 1-2 X 5 min is 1X PBS (both times on the oscillating table).

**Suuggestions:**

1. Unless specified, it is the same to block with milk or BSA. 2. You can store the membrane in 1X PBS after secondary antibody incubation at 4°C.