Integrated Systems Engineering S.r.l.
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# XBLOT P100

## x-Blot hybridization equipment

# XBLOT P100: description and advantages

The **XBLOT P100** is a motorized temperature-controlled roller intended for Western (W), Northern (N) and Southern (S) hybridizations as it is able to evenly distribute reagents of interest onto nitrocellulose or nylon (PVDF) filters containing electrophoresed or fractionated proteins (W), DNA (S) or RNA (N). The filters are enclosed within a perfectly sealed plastic envelope allowing the use of small amounts of reagents (just sufficient to keep the filter wet). The continuous movement of the roller facilitates the binding of the antibody to a protein (in case of Western Blot) or of a labelled probe to DNA or RNA (in case of Southern or Northern Blot).

## *Advantages in using as protein hybridizer XBLOT P100 over the standard method*

***(Analogous considerations may be as well made for DNA and RNA hybridization)***

- **Increase signal sharpness and reproducibility**

  The even distribution of reagents and the constant temperature during the hybridization process avoid precipitate formation and reagent evaporation, thus enhancing results acquisition.


**Classic** Hybridization time: Overnight

**XBLOT P100** Hybridization time: 15 min

- **Marked reduction in hybridization execution time**

- **Reduction in the amount of reagent used**

  Example:
  - 6 filters tested per day → total of 120 filters per month
  - Recovery of the solution containing the primary antibody and re-use for at least twice

  Total costs

  Plastic envelope hybridization using XBLOT P100 → 100.4€/month

  Classic hybridization in tray → 1010€/ month

  **Total money saving of about 900€/ month with XBLOT P100 hybridization!**

| **Plastic envelope hybridization Using XBLOT P100** | | **Classic hybridization In tray** |
|---|---|---|
| | Average volume of a filter 7x8cm | |
| ≤ 1ml | | ≥ 10 ml |
| | Average dilution **Primary antibody = 1:1000** Average cost per µl = 2€ | |
| Cost/Filter = 2€ | | Cost/Filter = 20€ |
| | Average dilution **Secondary antibody = 1:2000** Average cost per µl = 0,35€ | |
| Cost/Filter = 0,17€ | | Cost/Filter = 1,75€ |
| | **TOTAL COST/FILTER** | |
| **2,17 €** | | **21,75 €** |

- **Adjustable temperature according to reagent specifications**

**Other advantages**

- Reduction in the time necessary for blot developing.
- Marked recovery of the reagents due to the absence of evaporation.
- Possibility to handle several filters simultaneously.

# XBLOT P100: Western Blot applications

## Protocol

A typical protocol for Western hybridization using XBLOT P100 is reported.

1. Block the filter in 5% Non Fat Dry Milk-TBST (20mM Tris-HCl pH 7.6, 137mM NaCl, 0.1% Tween®20) for 1 hour at room temperature on a rocking platform shaker.
2. Place the filter in a plastic bag of slightly larger size than the membrane, add the primary antibody diluted in 5% Non Fat Dry Milk—TBST and seal. The suggested hybridization volume is 0.02 ml per $cm^2$ of filter.
3. Place the sealed bag onto the XBLOT plate, fix with sellotape and switch-on the instrument. The incubation time and temperature depend on the antibody used.
4. Wash the filter three times for 5 minutes each with TBST at room temperature on a rocking platform shaker.
5. Incubate the filter with the secondary antibody in TBST at room temperature for 1 hour.

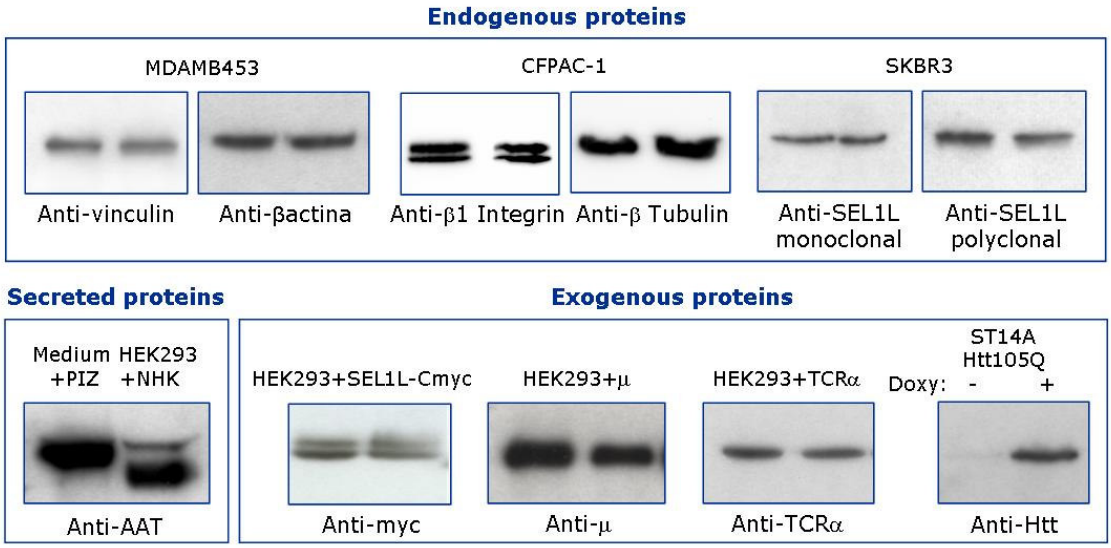
| Antibody | Temperature [°C] | Time [Hours] |
|---|---|---|
| Vinculin *(Sigma)* | 23-25 | 1 |
| β-actin *(Sigma)* | 23-25 | 1 |
| β1-integrin *(BD)* | 23-25 | 1 |
| β-tubulin *(Sigma)* | 23-25 | 15 min |
| SEL1L monoclonal *(Alexis)* | 23-25 | 1 |
| SEL1L polyclonal *(in house)* | 23-25 | 6 |
| AAT *(Sigma)* | 5 | ≤16 |
| myc *(Cell Signaling)* | 5 | 1 |
| *μ (Zymed)* | 23-25 | 6 |
| TCR-α *(SCBT)* | 5 | ≤16 |
| Htt *(Chemicon)* | 5 | ≤16 |

6. Wash the filter three times for 5 minutes with TBST at room temperature on a rocking platform shaker.
7. Detect the filter.

NB: Steps 2 to 6 included can all be performed on the XBLOT P100 instrument.

## Results

The panels below show some of the results obtained using cell lines and antibodies available. The results refer to endogenously expressed proteins as well as those induced to be expressed via transfection. Secreted proteins can be also detected.




*Upper panel*: Total cell extracts (30μg) from: MDAMB453, CFPAC-1 and SKBR3 cell lines loaded in duplicates.
*Lower right panel*: Total protein extracts (30μg) from HEK293 transfected cells loaded in duplicates. The exogenous Htt protein was detected after doxycycline treatment of the stably transfected cells ST14A/Htt105Q.
*Lower left panel*: detection of secreted variants of α1-antitrypsin (PIZ, NHK) obtained from the cell colture medium.

## Technical specification

- Temperature-controlled plate by Peltier module.
  Operating temperature:
  - 0*÷38°C for soft PVC coated roller
  - 0*÷70°C for black rubber coated roller

  * The minimum temperature depends on the room temperature. In the worst case, $\Delta T = T_{room} - T_{plate}$ greater than 20°C cannot be obtained.
- 20cm x 29.7cm plate to accommodate several filters simultaneously, also stratified.
- Adjustable roller speed.
- Adjustable roller travel distance according to the area occupied by the plastic envelopes.
- Steel roller mounted on radial ball-bearing and with soft PVC coating highly resistant to wear and chemical agents (Operating temperature: 0÷38°C).
- CE mark

## Optional accessories

**Black rubber coated roller *(Order Code: P-RubRol)***

- Steel roller mounted on radial ball-bearing and with black rubber sheath coating for use at higher temperature (Operating temperature: 0÷70°C).

**Plastic bags *(Order Code: P-PEbag)***

- Heavy-duty polyethylene film to be sealed with a thermal impulse sealer.

**Thermal impulse sealer**

- Not supplied with XBLOT P100.

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