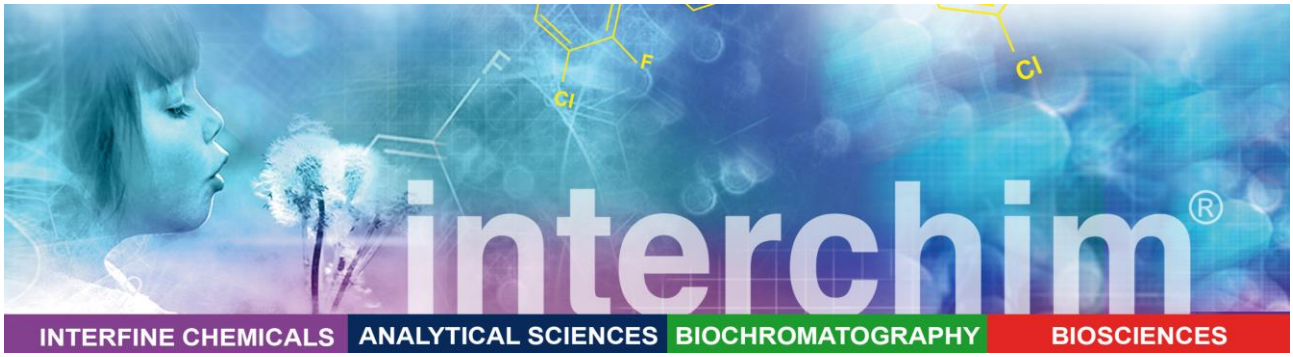
FT-L7710A



Antibody Stripping Buffer

Regenerate your blot in less than 20 minutes to re-probe it !

- Product Description

Name: **Antibody Stripping Solution**
Catalog number: L7710A, 500ml* GV0480, 500ml 5X
*Sufficient reagents for 3000 cm^2 or 200-400 membrane strips or 20-40 standard blots

- **Storage:** Store at RT (for a long storage: +4C°) (L)

- No pungent smelling mercaptoethanol
- Antibody stripping is done at room temperature. No heating of blots required
- Strip antibodies in just about 15 minutes at room temperature
- Reblocking of blots may be avoided in most instances

Save time
Saves costly sample
Economical

• Technical information

Western Blotting is a commonly used technique to study protein structure and functions: it detect and compare proteins from a complex mixture utilizing antibody detection. Typically, protein samples are electrophoresed on SDS-PAGE (separation according their molecular size or their isoelectric point), then transferred to solid support (immobilisation onto a nitrocellulose or nylon-based membrane) and finally probed with labeled antibodies.
Unlike advances made in similar techniques in probing nucleic acids (northern and southern blotting) that allow reuse of blots, it has been difficult to reuse blots for Western Blotting. Immuno-probing with chemiluminescence has become an easy and sensitive method of detection compared to other methods, notably colorimetric staining and even fluorescence detection. Furthermore, it is possible to 'recycle' blotted proteins for re-probing on the membrane. Western blots have been stripped using extremely harsh conditions that may alter the antigen for subsequent immunoprobing. Interchim provides a gentler and time-saving solution:

***Uptima* Antibody Stripping Buffer** is a novel formula that allows several re-probings on the same membrane.
It effectively and almost quantitatively removes primary and secondary antibodies from the blots without significantly affecting the immobilized proteins. This is a gentle method that allows several re-probings on the same membrane. It has been optimized for HRP chemiluminescence substrates in western blotting.

As a result, recycling of protein blots with Ab Stripping Buffer #L7710 is very useful and flexible:

- it is more economical and less time consuming to reuse the same blot than to prepare several blots
- for multiple detections on the same blot - save time and simplify and improve accuracy of probed blots overlay
- to spare protein samples that are available in limited quantities, difficult to obtain or expensive – only one blot!
- to analyze samples with different antibodies under identical conditions, e.g. subtype or isoform specific antibodies
- when the blot gives unexpected results, to confirm a result with the same or different antibody
- when a blot is mistakenly incubated with wrong antibody

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- to optimize antibody concentrations or to detect a new antigen with different antibodies

- Directions for Use

Procedure (Total stripping time: ~15 min)

The blots or individual strips that are to be re-probed should be prepared for stripping immediately after their first usage. If stripping cannot be performed right away, blots can be wrapped in Saran Wrap and stored moist in PBS or TBS at 4°C until stripping. DO NOT STORE BLOTS IN DRY FORM.

- Allow the Ab stripping buffer to reach room temperature
- Place the blot to be stripped in the Stripping Buffer. Add Stripping Buffer, to fully immerse the blot. Incubate the blot in the Striping buffer for 5-15 min at room temperature with strong shaking.

Note: In general, higher affinity antibodies or large quantities of detected protein will require longer incubation time for stripping.

- To wash, empty the Stripping Buffer, add 300 ml of dH_2O and shake vigorously.
- Repeat Steps 3 five more times with blocking solution. The blot is now ready for reprobing with antibodies.

Note1: The blot is now ready for reprobing with antibodies, unless:
-one wants to perform a new blocking step, needed to reduce significant background in you application, using your own blocking and probing protocol. See 'Additional information.
-one wants to perform checks: see note 2/optionnal steps 5 and 6).

Note2: The blot can be stripped several times (up 5-10 times). However, longer exposure times or more sensitive antibody detections may be required. Actually, repeated blotting and stripping processes may affect antigens. Analysis of the individual system is required.

Procedure for stripping checks

In case background or unexpected proteins have been detected during a stripped-blot reprobing, it is recommended to check for complete removal of the HRP label (optional step 5) and eventually complete removal of primary antibody (optional step 6):

- (optional: checking the complete removal of the HRP label monitoring).
  Incubate the stripped blot from step 4 with fresh chemiluminescent substrate (UptiLight ECL). Wash well for further use.

- (optional: checking the complete removal of primary label antibody monitoring)
  Incubate the stripped blot from step 4 with HRP-labeled II antibody, then wash and proceed as in step 5.

If signal is detected in the steps 5 or 6, place the blot back into Stripping Buffer for additional 5-15 min, and continue to step 3.
If no signal was detected, wash well the blot with PBS and a new blotting procedure can be performed.

- Additional information and Suggestions

This kit is primarily designed to allow investigators to strip previously bound primary antibody and enzymes conjugates. This kit has been optimized to be used with Chemiluminescent substrate kits (ask our UptiLight substrate #98490B). This kit can be used to strip antibodies that were labeled with radioactive iodine or other isotopes. **It is not recommended for procedures that use color development** (TMB, 4-Chloronaphtol, etc) as it is not possible to completely remove the substrate that precipitates at the reaction site.

The stripping procedure works very well in most cases, with any ag/ab systems, that is to say protein/antigens are not affected and I and II abs are completely removed . However it may be useful for specific applications to check with proper positive and negative controls that the stripping and reprobing are effective. It is i.e. possible in specific cases that some antigens were affected or labile, and ag/ab or probes binding are not completely broken because of very high affinity (i.e. biotin/avidins).
First, a **positive and a negative sample** should be included in the western-Blot.
The **right stripping** may be checked, as proposed in procedure 'stripping checks'.

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One fresh blot strip (not processed with stripping solution) may be processed during the second blot staining to check **proper staining conditions**.

This kit should only be used for qualitative purposes unless it has been established that recycling does not quantitatively affect a given antigen.

It is generally not necessary to reblock with blocking solution after each stripping cycle. However, if excessive background occurs after stripping, we recommend that blots be reblocked with blocking buffer, i.e. with milk based solution or SeaBlock #UP4030, or any other suitable buffer. It is also suggested that the user employs its own blocking and probing protocol.
Nylon based membranes in general require either longer incubation or higher concentration of blocking agent (e.g., 10% BSA/milk instead of 5% with nitrocellulose). Therefore, blocking conditions that are known to work with a given membrane can be used after stripping.
Some antibodies do not work very well with milk based blocking agents. It is suggested that another blocking agent that is known to work with a given antibody be tried after each stripping. Blocking with certain agents (e.g. albumin) may lead to higher background upon stripping. Re-blocking with the supplied blocking buffer may help reduce background.

This reagent is sold for in vitro research use only.

- Related products

Western blot products[]

- Mix-n-Stain HRP Antibody Labeling Kit, PQI240
- ProTran 0.2µm NC membranes, 20x20cm, S31441
- Blotting paper 1mm thick, 460x570cm, BP2791
- Protein Membrane Reversible stain, 20078A
- Non fat milk powder, 768701
- SeaBlock agent, UP40301A
- TBS with non fat milk, GS4162
- BSA Biotech grade, UPQ84170 (powder) or UP900130 (solution 30%)
- BioBlock Saturating agent for blotting, N13650
- UptiLight HRP WB
  High Sensitivity Substrate, 98490B
  Ultra Sensitivity Substrate, 58372B
  One component Substrate, Spray BM4961, Dropper BM4963

- Ordering information

Catalog size quantities and prices may be found at http://www.interchim.com.

For any information, please ask : Uptima / Interchim; Hotline : +33(0)4 70 03 73 06

Order on-line or Contact your local distributor

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