

Detection of Proteins on Blot Transfer Membranes

In the three protocols described below, proteins are stained after electroblotting from polyacrylamide gels to blot transfer membranes. If the samples of interest are electrophoresed in duplicate and transferred to a blot transfer membrane, half of the membrane can be stained to determine the efficiency of transfer to the membrane and the other half can be used for immunoblotting (i.e., Western blotting; see UNIT 10.8). Table 10.7.1 below provides detection limits of each staining method and compatible blot transfer membranes and gels.

Table 10.7.1 Properties and Compatibilities of "On Blot" Membrane Stains

Stain	Detection limit (ng)	Membrane types ^a			Gel types	
		Nitro-cellulose	Nylon	PVDF	SDS-PAGE	Native PAGE
India ink	50	+	-	+	+	+
Gold	3	+	-	+	+	?
Iron	25	+	+	+	+	-

^aAbbreviations: PVDF, polyvinylidene difluoride; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

BASIC PROTOCOL

INDIA INK STAINING

India ink is used to stain proteins on blot transfer membranes. The transferred proteins (≥ 50 ng) appear as black bands on a gray background.

Materials

- Tween 20 solution
- India ink solution
- Plastic boxes

NOTE: *Deionized, distilled water should be used throughout this protocol.*

1. Place blot transfer membrane(s) in a plastic box on an orbital shaker and wash in Tween 20 solution at 37°C three times for 30 min each.
2. Continue to wash the membrane in Tween 20 solution at room temperature two times for 30 min each.
3. Stain the membrane in India ink solution 3 hr or overnight.
4. Rinse the membrane twice in Tween 20 solution and destain in Tween 20 solution until an acceptable background is obtained, then air dry.

Black bands appear against a gray background.

ALTERNATE PROTOCOL

GOLD STAINING

A colloidal gold sol is used to stain proteins on blot transfer membranes. The transferred proteins (≥ 3 ng) will appear as red bands on an almost white background.

Additional Materials

- AuroDye colloidal gold sol (Janssen Pharmaceutica)
- Heat-sealable plastic bag

1. Place blot transfer membrane(s) in a plastic box on an orbital shaker and wash in Tween 20 solution three times at 37°C for 30 min each.

Do not attempt to stain nylon membranes.

2. Continue to wash the membrane in Tween 20 solution at room temperature two times for 30 min each.
3. Stain the membrane with continuous shaking in AuroDye colloidal gold sol for 3 hr or overnight.
A heat-sealed plastic bag is a convenient container for the staining.
4. Rinse the membrane briefly in water and air dry.

IRON STAINING

A colloidal iron sol is used to stain proteins on blot transfer membranes. The transferred proteins (≥ 25 ng) will appear as dark blue bands on an almost white background.

Additional Materials

FerriDye colloidal iron sol (Janssen Pharmaceutica)
FerriDye developer (Janssen Pharmaceutica)

1. Place membrane transfer blot(s) in a plastic box on an orbital shaker and wash in water three times for 10 min each.
2. Stain in FerriDye colloidal iron sol 1 to 2 hr.
3. Rinse in water three times for 2 min each.
4. Develop in freshly prepared FerriDye developer for 1 min.

CAUTION: Due to the release of poisonous hydrogen cyanide gas the preparation of the developer and the incubation must be carried out under a fume hood.

5. Rinse several times in water and air dry.

REAGENTS AND SOLUTIONS

NOTE: *Deionized, distilled water should be used to prepare all solutions.*

Tween 20 solution

0.3% (vol/vol) Tween 20 in phosphate-buffered saline (PBS; *APPENDIX 2*), pH 7.4.

India ink solution

0.1% India ink (e.g., Pelikan 17 black) in Tween 20 solution.

COMMENTARY

Background Information

The introduction of the electrophoretic transfer of separated proteins has been paralleled by the use of extremely sensitive overlay techniques to detect the binding of antibodies and lectins to the transferred protein bands. "On-blot" protein staining procedures help one control the electrophoretic transfer and correlate the transferred polypeptide pattern with that detected in the overlay. An additional advantage of on-blot protein staining as compared to gel staining is that the pattern of the protein membrane stains can be directly compared to the results of the overlay assays. Comparing overlay assays with stained gels

is cumbersome due to swelling or shrinking of the polyacrylamide gels.

Membranes used for the electrophoretic transfer are manufactured from nitrocellulose, nylon, or polyvinylidene difluoride (PVDF). Depending on the transfer membrane used and other considerations (e.g., the use of SDS-polyacrylamine gels or polyacrylamide gels without SDS and the level of sensitivity required), different staining procedures must be selected (Table 10.7.1).

The staining of membrane-bound polypeptides by gold sol is mediated by hydrophobic interactions and by ionic interaction of negatively charged gold particles with posi-

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tive groups on the proteins. The protocol listed is that described by Moeremans et al. (1985). The gold stain is very easy to perform and is the most sensitive blot membrane stain.

The staining of membrane-bound polypeptides by positively charged iron sol is about 8-fold less sensitive than the reaction with gold sol. However, iron sol is the preferred staining technique for proteins transferred to positively charged nylon membranes. The protocol is that described by Moeremans et al. (1986).

The protocol for India ink staining of polypeptides is based on the procedure described by Hancock and Tsang (1983).

Critical Parameters

It is mandatory that all glassware and plasticware used with the gels and blot membranes be thoroughly cleaned in order to avoid staining artifacts. All blot membranes should be handled by forceps only. The gold stain cannot be used for detecting polypeptides blotted onto nylon membranes, since these membranes are positively charged. Furthermore, due to its very high sensitivity the gold stain is susceptible to impurities present in buffers and on the surface of staining boxes.

Anticipated Results

Detection limits and compatibilities of transfer membranes and gel types are summarized in Table 10.7.1.

Literature Cited

- Hancock, K. and Tsang, V.C.M. 1983. India ink staining of proteins on nitrocellulose paper. *Anal. Biochem.* 133:157-162.
- Moeremans, M., Dancels, G., and De Mey, J. 1985. Sensitive colloidal metal (gold or silver) staining of protein blots on nitrocellulose membranes. *Anal. Biochem.* 145:315-321.
- Moeremans, M., De Raeymaeker, M., Dancels, G., and De Mey, J. 1986. FerriDye: Colloidal iron binding followed by Perls' reaction for the staining of proteins transferred from sodium dodecyl sulfate gels to nitrocellulose and positively charged nylon membranes. *Anal. Biochem.* 153:18-22.

Key References

- Moeremans et al., 1985 and 1986. See above.
- For investigators wishing to prepare their own reagents, these papers include descriptions of how to make gold and iron sols.*

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