

# A sensitive and simple procedure for staining proteins on either nitrocellulose or PVDF membranes based on the fluorophore epicocconone.

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## introduction

Identification of biomarkers using immuno-staining of proteins transferred to a membrane using Western blotting is a widely used technique.

Total protein quantification of transferred proteins is important in order to:

- Assess the level of expression of the protein(s) of interest.
- Most immuno-staining methods are poorly quantitative.
- Ensure efficient transfer before undertaking time-consuming immuno-staining.
- Visualise the level of expression of the protein(s) of interest against the background of other proteins.

Currently available total protein stains for blots lack the linear dynamic range and sensitivity required or interfere with subsequent immuno-staining.

LavaPurple™ is a new total protein stain based on the novel molecule epicocconone that is suitable for staining gels, nitrocellulose and PVDF membranes. The unique reversibility epicocconone binding<sup>1</sup> led us to investigate its suitability for protein quantification on Western blots prior to immuno-staining.

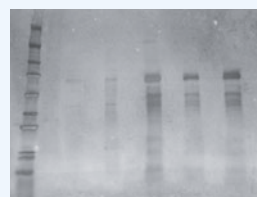
## methods

- OvCa 429 cell lysate and conditioned media were used as the protein samples.
- 1DGE was performed using 4-20% Tris-HCl mini-gels (BioRad) or 12% Bis-Tris mini-gels (Invitrogen).
- Proteins were transferred onto PVDF membrane in an Invitrogen XCell™ trans-blotter following the manufacturer's protocol.
- LavaPurple blot staining was performed as per the manufacturer's instructions.
- De-staining of LavaPurple was performed in 50% MeCN in 30 mM NH<sub>4</sub>HCO<sub>3</sub> pH 8.5.
- Immuno-staining was performed using the 6.2A1 primary antibody (kindly provided by P. Weinreb of Biogen Idec Inc.) and ECL-Plus™ kit (GE-Healthcare) secondary antibody labeling.
- LavaPurple imaging was performed using a Typhoon™ 9200 imager (GE-Healthcare) with a 532 nm laser, 610-30 BP filter, 400 PMT, 100 μm resolution, and normal sensitivity.
- Imaging of the antibody labeling was performed using a Fuji LAS-3000 imaging system.
- Volume analysis of Bands was performed using ImageQuant TL™ image analysis software.

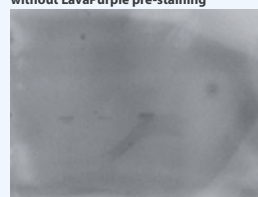
## results

- Staining of gel after transfer exhibited protein bands in all the dilution lanes, illustrating the lack of complete transfer.
- LavaPurple staining of the blot exhibited bands in both the cell lysate and cell culture supernatant lanes.
- After de-staining the membrane for 15 minutes the residual LavaPurple signal was reduced to 30%. This was further reduced to 0.6% by de-staining overnight.
- Re-staining of the blots resulted in similar signal strength indicating protein was not removed from the blots.
- LavaPurple did not significantly increase the background of the PVDF membrane when subsequently blocked, and stained with the GE ECL-Plus kit.

LavaPurple stained membrane



ECL-Plus immuno-stained membranes without LavaPurple pre-staining



ECL-Plus immuno-stained membranes with LavaPurple pre-staining



**Figure 1.** Membranes that have been probed with a primary antibody and labeled with a chemi-luminescent secondary antibody, both with and without pre-staining with LavaPurple.

LavaPurple Staining Characteristics	
Residual LavaPurple signal after 15 minutes de-staining	30.0%
Residual LavaPurple signal after overnight de-staining	0.6%
Average relative signal after re-staining with LavaPurple	110.5%
Relative signal of bands immunostained after total protein staining	144.1%
Relative Background ECL signal of pre-stained blot	100.1%

**Table 1.** Comparison of Western blots on PVDF membranes probed with ECL-Plus kit either with or without pre-staining with LavaPurple.

## conclusion

LavaPurple Total Protein Stain is suitable for quantification of proteins Western blotted on to PVDF. The stain is simple to remove leaving proteins unmodified that are suitable for subsequent immuno-staining.

LavaPurple has been shown to be suitable for staining proteins transferred to nitrocellulose or PVDF membranes, from 1DGE or 2DGE, and has sensitivity in the order of 1 ng protein<sup>2</sup>.

This sensitivity combined with its compatibility with immuno-staining makes LavaPurple Total Protein Stain particularly suitable for blot staining applications including the QC of antigens/antibody kits, or the comparison of biomarker expression levels where the amount of sample available for blotting is limited.

## references

1. Coghlan, D.R., *et al.* (2005) Mechanism of reversible fluorescent staining of protein with Epicocconone. *Organic Letters*, 7, 2401-2404.

2. Malmport, E., Mackintosh, J., Ji, H., Veal, D. & Karuso, P. (2005) Visualization of proteins electro-transferred on Hybond ECL and Hybond-P using Deep Purple Total Protein Stain. *GE-Healthcare Life Science News*, 19, 12-13.

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