

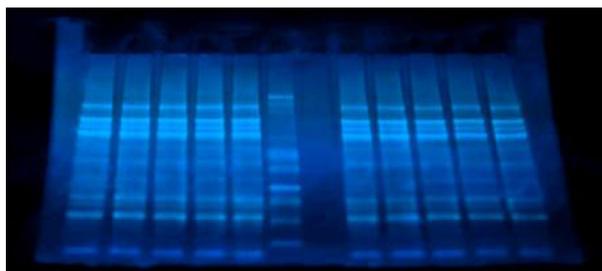
nUView Precast Gels

UV VISUALIZATION



All nUView precast gels incorporate a unique formulation allowing protein bands to be visualized in only 2 minutes in the presence of ultraviolet (UV) light - taking you from analysis to results faster than any other gel on the market!

With nUView technology you no longer have to stain and de-stain your gels – increasing protein recovery and saving you significant time while achieving improved results.



2 minute visualization under UV light

Visualization of Proteins in SDS-PAGE nUView System

SDS-PAGE (Sodium Dodecyl Sulphate – Polyacrylamide Gel Electrophoresis) is possibly the most common and long standing analytical tool used in protein chemistry [Laemmli, 1970].

The method is used to separate proteins according to their electrophoretic mobility which in SDS-PAGE is a function of length of polypeptide chain or molecular weight due to the proteins in the samples having near identical charge per unit mass due to the binding of SDS.

Visualisation commonly uses textile dyes such as Coomassie Brilliant Blue (CBB). These and other approaches such as silver staining, fluorescent dyes and heavy metal binding are protracted methods of staining. Most of these methods utilize either expensive reagents, cause problems with waste disposal or require specialized imaging systems.

UV Visualization

In contrast, nUView gels allow the separated protein bands to be visualized by illuminating the gel on a standard UV trans-illuminator such as that commonly used in molecular biology laboratories for the visualization of DNA with interchelating dyes (e.g., ethidium bromide).

The amino acid tryptophan is naturally fluorescent, but not in the visible spectrum. Photo-induced adduct formation between the tryptophan and the trihalo-compound, under UV illumination, results in proteins becoming fluorescent in the visible range, with excitation by UV light.

Optimal results are dependent on the trans-illuminator wavelength, the filters in place and the camera system used to document the fluorescence. Bands can be seen within 2 minutes of UV exposure. Therefore, one needs to optimize the conditions for any given gel documentation system and UV trans-illuminator.

Increased exposure times may lead to increased fluorescence intensity. However, cumulative UV exposure times will alter the tryptophan adduct to a nonfluorescent form, thus the protein bands dim (starting after approx. 7 minutes) and eventually are no longer visible under UV illumination.

Process Optimization

Use a UV trans-illuminator with UV tubes that provide a wavelength in the range 250-320nm. 300-310nm is preferred but the others can be optimized. To determine the optimal illumination time, run a gel with a range of loading concentrations of a commonly used sample or molecular weight standards. Remove gel from the cassette, rinse briefly with RO water (do not soak the gel) and place directly onto the trans-illuminator (rinsing can be omitted, however the salts in the surface buffer on the gel may be corrosive for parts of the trans-illuminator). Turn the UV light on and expose gel for 2 minutes. It may be possible to monitor the gel development in real time.

Optimize your camera parameters (F stop, focus, etc). At 2 minutes and regular intervals up to 10-15 minutes (at the longer time you may notice a decrease in intensity) take photos/images of the exposed gel. Compare the pictures and select the optimal detection time.

Applications

The nUView method provides benefits in electroblotting to evaluate/document a gel prior to Western blotting [Ladner et al., 2004] and autoradiography; as it does not require a replicate gel to be run and, after blotting, the original gel can be placed back on the transilluminator to evaluate the degree of protein transfer to the membrane.

Bands visualized by nUView can be excised and analysed by mass spectrometry. The bands visualized with the nUView method could be digested and applied to MALDI/TOF plates directly [Ladner et al., 2006]. Proteins must be fixed before being digested and eluting peptides from the gel for LC-MS analysis.

nUView visualized gels can be over-stained by any preferred method - CBB, silver, Sypro, zinc/copper, etc. Thus the nUView method provides instant visualization, which then can be followed by standard imaging techniques. Furthermore, this combination provides a more comprehensive picture of all proteins in the sample as not all proteins are necessarily detectable by a single visualization method. Membrane proteins may be more easily visualized with nUView versus CBB due to tryptophan content of this class of proteins.

References

Ladner, C. Yang, J., Turner, R.J., & Edwards, R. A. (2004) Visible Fluorescence Detection of Proteins in Polyacrylamide Gels without staining. *Anal. Biochem.* 326;13-20.

Ladner, C.L., Edwards, R.A., Schriemer, D.C., & Turner, R.J. (2006) Identification of trichloroethanol visualized proteins from two-dimensional polyacrylamide gels by mass spectrometry. *Analytical Chemistry.* 78; 2388-2396.

Laemmli, U.K. (1970) Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4. *Nature* 227; 680-685. G0311D