

product code

RPN6305

RPN6306

Deep Purple Total Protein Stain

Warning

For research use only.

Not recommended or intended
for diagnosis of disease in
humans or animals.

Do not use internally or
externally in humans or
animals.



Handling

Storage

On receipt, store in a freezer at -15 °C to -30 °C.

Expiry

The product is stable for at least 3 months when stored under the recommended conditions. Deep purple total protein stain is sensitive to prolonged exposure to light. Long term storage of the reagent should be in the light tight container in which it is provided.

Components

This pack contains the following

- RPN6305
Deep Purple Protein Stain, 5 ml
Reconstitutes to 1 litre.
- RPN6306
Deep Purple Protein Stain, 25 ml
Reconstitutes to 5 litres.

Solution supplied contains Deep Purple total protein stain in 50% (v/v) DMSO and 50% (v/v) acetonitrile.

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Safety warnings and precautions

Warning: For research use only. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

As all chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques, and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See MSD(s) and/or SS(s) for specific component handling instructions.

Note: The protocol requires the use of acetic acid, methanol and ammonia solution.

Warning: Acetic acid causes burns and is an irritant. Please follow the manufacturer's safety data sheet relating to the safe handling and use of this material.

Warning: Methanol is toxic and flammable.

Please follow the manufacturer's safety data sheet relating to the safe handling and use of this material.

Warning: Ammonia solution is an irritant. Please follow the manufacturer's safety data sheet relating to the safe handling and use of this material.

Note: The protocol may involve the use of UV illumination devices and / or laser based imaging instruments.

Warning: In the case of both UV illumination or laser scanning it should be ensured that proper and effective safety regulations are followed. When using UV illumination, a full face UV protective visor should be worn.

Description

Deep Purple™ total protein stain is a naturally occurring fluorescent compound extracted from a fungal species (1) and it has been developed as an ultra-sensitive fluorescent stain for the detection of proteins in-gel following electrophoretic separation (2). It is believed that this stain interacts with SDS micelles surrounding proteins within gels although a detailed mode of action is not yet fully established. Deep Purple has been shown to be up to 8 times more sensitive than similar products although, as with all protein staining methods certain individual proteins can exhibit different staining characteristics. Deep Purple is an environmentally friendly stain which is diluted in water for use and therefore allows for easy and convenient disposal. The stain may be used in conjunction with a range of excitation sources including UV light boxes, broad range visible light sources and lasers (see figure 1).

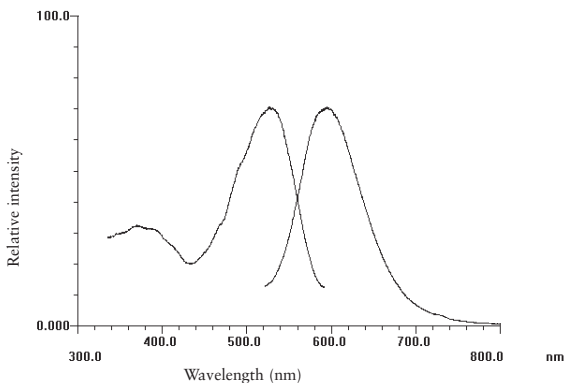


Figure 1. Fluorescence excitation and emission spectra of Deep Purple stained proteins in gel plugs taken from a 2D gel.

Procedure

Deep Purple is simple to use and reliable. Gels are run and then fixed in standard conditions using, for example, an acetic acid / methanol solution. A staining protocol is provided for use with both free-floating gels and for gels immobilized on glass plates. The stain can therefore be used for both standard 1D and 2D gel electrophoresis.

The stain is stable as supplied at $-15\text{ }^{\circ}\text{C}$ to $-30\text{ }^{\circ}\text{C}$ for at least 3 months and it has not been observed to generate particles or sediment over time. Working solution (1:200 dilution) is stable at $2\text{--}8\text{ }^{\circ}\text{C}$ for up to 1 week and 24 h at room temperature, while stained gels can be stored for many weeks without significant loss in sensitivity. In addition, if some signal is lost, it is possible to re-stain gels and gain virtually the same intensity signal.

The stain protocol allows for flexibility within individual experimental work-flows, providing a rapid protocol of less than 3.5 hours for speedy results or an overnight fix step for maximum sensitivity and work-flow convenience.

Applications

The stain can be used on pre-cast or lab-cast, Bis-Tris and Tris-Glycine gels; with gel systems containing low or high SDS levels; and is compatible with other buffer systems such as MES, MOPS or tricine.

Deep Purple stained gels can be imaged on a variety of instruments. Flat bed laser based fluorescence scanners are strongly recommended for optimal imaging of Deep Purple stained gels, for example using the Typhoon™ scanner. However the stain can also be visualized with UV illumination at 302 nm or 365 nm and images can be captured on a suitable CCD or video camera system.

Deep Purple fits into the standard 2D gel electrophoresis work-flow and is particularly suitable for use with the Ettan™ DIGE system. The

recommended work-flow for this system involves the matching of Deep Purple stained preparatory gels with CyDye™ labeled analytical gels. Deep Purple has been shown to be compatible with DeCyder™ difference analysis software and the stain is compatible with manual or automated spot picking and mass spectrometry for protein identification applications.

Critical parameters

- Read the entire protocol thoroughly before using the kit.
- Ensure that the containers used for gels are clean and do not contain any contaminants. A wide variety of non-metallic containers can be used with this stain, including polypropylene, polystyrene or Pyrex™ glass.
- It is essential to ensure that plates to be coated with Bind-Silane are prepared to the highest standard (see ‘Additional Information’, page 18).
- It is recommended to use gloves that are not powdered. Wash new gloves prior to handling plates, containers or gels. Any powder transferred to the gel may show up as speckles on images.
- During preparation of plates for gel casting, it is advised to employ methods that minimize generation of dust particles. The use of any type of paper towel will generate particulate matter that will be visualized as ‘speckles’. Plates should be cleaned using lint free cloths, such as Crew™ Wipers.

- During the protein staining step a volume of working stain solution equivalent to at least a 10-fold excess of the gel volume should be used. During the fixation step, gel washes and ammonia incubations a volume equivalent to ~25-fold excess of the gel volume should be used (see Table 1, below).

Table 1. Typical stain and gel processing solution volumes for the Deep Purple total protein stain protocol.

Electrophoresis System	Gel Dimensions (cm)	Stain Volume (ml)	Processing Solution Volume (ml)
Hoefer™ miniVE	10 x 10 x 0.05	50	120
Hoefer SE 260	10 x 10 x 0.05	50	120
Hoefer SE 400	18 x 16 x 0.1	250	600
Hoefer SE 600	18 x 16 x 0.1	250	600
Hoefer SE 660	18 x 24 x 0.1	500	1200
Ettan DALT <i>six</i>	20 x 26 x 0.1	500	1200
Ettan DALT <i>twelve</i>	20 x 26 x 0.1	500	1200

- Do not dilute the stain beyond 1:200 as this will result in reduced intensity and sensitivity.
- Do not re-use the stain solution as this may result in a significant loss of sensitivity.
- During the process gel containers should be covered to exclude light and agitated gently on a mixer platform.

- The source of SDS used to prepare and run polyacrylamide gels can affect the background obtained on imaging. It is recommended that high quality materials are used such as those supplied by BDH and USB. Certain commercially available pre-made running buffers may not be suitable, particularly when using short fixation times.

Solutions and reagents required

Amersham Biosciences reagents

SDS : USB reagent (US75819-100g or US85819-1kg) or similar high quality material (such as BDH Specially Pure grade).

Acrylamide gel and other related electrophoresis reagents are available in the PlusOne™ range and are detailed in the current BioDirectory™ catalogue.

Additional reagents required

High purity water (double distilled, RO or equivalent).

Acetic acid, glacial.

Concentrated ammonia solution is commercially supplied as 25% w/v NH₃ in water and has a specific gravity of 0.88 - 0.91.

Solutions required

Note: High purity water (RO quality or better) should be used as a diluent for Deep Purple total protein stain, for gel wash steps as indicated in the protocol and for preparing all gel processing solutions.

Fixation solution

7.5% (v/v) acetic acid / 10% (v/v) methanol.

Add 75 ml acetic acid and 100 ml methanol to 825 ml water.

Working stain solution

1 in 200 dilution of Deep Purple stock solution in water.

For the appropriate volume to use refer to Table 1 (see page 8). This solution should be made fresh at the time of use. If necessary it is possible to store this solution, protected from exposure to light, for up to 1 week at 2-8 °C or 24h at room temperature.

Stain development solution

1 in 1000 dilution of concentrated ammonia solution in water.

Prepare an ammonia stock solution by diluting the concentrated ammonia solution 100-fold in water. This can be achieved by adding 10 ml of concentrated ammonia solution to 990 ml water. This stock solution can be stored for up to 1 month at room temperature in a well sealed bottle.

A 1 in 1000 dilution of concentrated ammonia solution is used to develop the stain. To prepare the Stain Development Solution, add 100 ml of ammonia stock solution to 900 ml water. This solution should be prepared fresh at each time of use.

Stabilization solution

0.75% (v/v) acetic acid.

Make a 7.5% acetic acid stock solution by adding 75 ml glacial acetic acid to 925 ml water. Add 100 ml 7.5% acetic acid stock solution to 900 ml water to form the stabilization solution.

Protocol

1 Gel electrophoresis

1.1 Perform electrophoresis according to established techniques.

Note: If visual orientation is required on 1D gels, Rainbow™ Markers (RPN800) may be used. If a tracking dye is used, such as bromophenol blue in the loading buffer, it is recommended to run the dye front just off the bottom of the gel.

2 Fixation

2.1 Place an appropriate volume of 7.5%(v/v) acetic acid / 10%(v/v) methanol into the containers that will be used to process gels. The recommended volume of fixation solution required is ~25 fold excess of the gel volume (see Table 1, page 8).

Note: Alternative fixation solutions that have been used successfully with Deep Purple total protein stain are:

- 7.5% acetic acid
- 7.5% acetic acid / 10% ethanol
- 7.0% acetic acid / 30% ethanol

It is recommended that a fixation solution, containing either ethanol or methanol, be used for any system that contains high levels of SDS.

2.2 Dismantle the apparatus.

- For free floating gels remove the gel from the plates by floating the gel off with gentle agitation in the fix solution.
- For backed gels place the gel and plate directly into the fix solution.

Note: Place only one gel in each container. The stacking gel can be left attached to help with gel orientation.

2.3 Incubate in the fixation solution, for a minimum of 1 hour, at room temperature with gentle agitation.

Note: It is recommended for backed gels and thick gels (≥ 1.5 mm) that an overnight fixation is performed. Overnight fixation is also recommended for applications where maximum sensitivity is required.

3 Staining

3.1 Pour off the fixation solution and replace with high purity water. Wash with gentle agitation for 4×10 minutes using fresh changes of water for each wash.

Note: During the fourth wash, take the stain out of the -15 °C to -30 °C freezer and allow to stand at room temperature for 2–3 min.

3.2 A few minutes before the fourth wash finishes, briefly shake the stain concentrate, measure out a suitable aliquot of the concentrated stock stain and dilute 1:200 with high purity water. The volume of working stain solution required is a 10-fold excess of the gel volume (See Table 1, page 8).

Note: The solution may turn yellow at this point or during the subsequent incubation – this is entirely normal.

3.3 Pour off the last water wash and replace with the 1:200 stain dilution. Cover the container to create a dark environment and incubate for 1 hour at room temperature with gentle agitation.

Note: Containers can be wrapped in foil or covered with black plastic. It is not necessary to eliminate light completely, only to ensure that bright light is significantly reduced. Alternatively, containers, with lids, that are a solid colored plastic may be used.

3.4 Pour off the stain and replace with freshly prepared Stain Development Solution. Cover the container to create a dark environment. Incubate with gentle agitation for 3×10 minutes using fresh changes of Stain Development Solution.

Note: Use the same volume as used for the fixation step. Stain Development Solution is a 1000-fold dilution of commercially available concentrated ammonia.

3.5 Pour off the last ammonia wash and replace with 0.75%(v/v) acetic acid. Cover the container to create a dark environment and incubate with gentle agitation for at least 10 minutes. The gel can be imaged at this stage.

Note: Further washes in 0.75%(v/v) acetic acid can be performed to reduce background still further if necessary. After imaging, the gels can be stored in the dark in 0.75%(v/v) acetic acid at 2–8 °C for several weeks. This allows for further imaging at a later date if required.

4 Visualization

4.1 Flat-bed laser based fluorescence imaging systems

4.1.1 Ensure that the scanning bed of the laser is clean and free from smears and particles. Follow recommended procedures provided with the instrument.

Note: On the Typhoon scanner it has been shown that fluorescent contamination on the platen can be eliminated by wiping the surface with 10%(v/v) H₂O₂ (hydrogen peroxide) followed by a rinse with double distilled water (see ‘Additional Information, page 17 for full details).

4.1.2 Set up the scanner as recommended in the relevant system operational manual.

For example, the following settings are recommended for use with a Typhoon scanner;

Excitation : Green laser (532 nm)

Emission: 560LP general filter

PMT: 550 V.

Pre-scan using 1000 micron resolution and then scan using a 100 micron resolution.

Note: If the pre-scan shows saturated bands/spots, reduce the PMT voltage rating and pre-scan again. If the pre-scan shows too low signal increase the PMT voltage rating and pre-scan again. Deep Purple can also be imaged on a Typhoon scanner using the blue laser (457 nm or 488 nm). If using an alternate fluorescent scanner, for the best optimal images, scan using as similar settings as possible to those recommended.

4.1.3 Process the image according to experimental requirements and the instructions for the relevant software program.

4.2 Imaging with UV light sources

4.2.1 Place the gel onto the UV transilluminator (302 or 365 nm wavelength emission required) and follow the operating and safety instructions as relevant for the excitation instrument and imaging system. Images can be captured using appropriate camera systems and

filters (film, video, CCD).

Note: For long periods of illumination it is advisable to place the gel on a glass plate, raised on spacers above the transilluminator, in order to reduce heat damage to the stained proteins. Cooling the gel prior to visualization can also help reduce degradation.

4.2.2 If required, pick any bands / spots.

Note: If manually picking bands / spots, it is advisable to place gels on a glass plate in order to reduce possible damage to the instrument surface. Prolonged, continuous exposure to a strong UV light source will degrade the Deep Purple signal, with a half-life in the region of 15 to 30 minutes

Additional information

Re-staining of gels

Gels that have lost sensitivity over time or through incorrect storage can be re-stained. In addition gels can be photo-bleached by prolonged exposure to UV light and then re-stained. In all cases the main protocol should be followed starting at the 'Staining' process (step 3, page 12) but only a single water wash is necessary to rinse away the acetic acid stabilization solution.

Alternative imaging instruments

Imaging is best performed using laser scanning fluorescence flat bed imaging systems, such as the Typhoon imager. This instrument can be used to excite Deep Purple at 457 nm, 488 nm or 532 nm with emission being collected through a 560 nm long pass filter or a 610 nm band pass filter. When using alternative laser based fluorescence scanners select laser wavelength and emission filters that are closest to those presented in the main protocol. Optimization of the scanning process may be required to account for the relative power of different lasers and the use of alternative filter settings.

Many makes of UV transilluminator are available that produce light of either 302 nm or 365 nm wavelength and both wavelengths are compatible with gels stained with Deep Purple. Gels excited by UV light can be visualized in a number of ways but if further image analysis is required it is recommended that the image is captured on a CCD camera system, such as the BioChemi™ Darkroom (UVP); the ethidium bromide (610 nm band pass) emission filter has been used to give satisfactory images from Deep Purple stained gels.

An alternative is the Dark Reader™ (Clare Chemical Research) which excites with blue light of wavelength ~430–480 nm. This is sufficiently

near the excitation peak of Deep Purple to produce useful fluorescence although a plain amber filter is recommended to exclude light of blue wavelength when imaging the gel.

Use of Deep Purple with Ettan DIGE

Deep Purple fits into the standard 2D gel electrophoresis work-flow and is particularly suitable for use with the Ettan DIGE system. The recommended work-flow involves the matching of Deep Purple post-stained preparatory gels with CyDye pre-labeled analytical gels. However, if it is necessary to post-stain analytical gels with Deep Purple, it is recommended to image the protein stain using 457 nm laser excitation in conjunction with a 610 nm band pass emission filter (or equivalent if not using a Typhoon scanner). This will minimize any potential cross-talk between Deep Purple and the CyDye DIGE fluors. Deep Purple has been shown to be compatible with DeCyder™ difference analysis software and the stain is compatible with manual or automated spot picking and mass spectrometry for protein identification applications.

Cleaning of imaging instruments

Deep Purple total protein stain may leave a fluorescent residue on the scanner platen. If the platen is not thoroughly cleaned, this residue can interfere with subsequent scans producing high background levels.

The following cleaning procedure has been shown to be compatible with the Typhoon fluorescent imager to remove contamination caused by fluorescent products. Compatibility of this procedure with alternative instruments would need to be investigated.

- Wipe the platen with 10% H₂O₂ (hydrogen peroxide).
- Rinse the platen with high purity water.
- This operation should be carried out using lint free cloths, such as Crew Wipers. A pre-scan can be done to check for contaminants that

may affect results of scans.

- If the scanner is shared and used for scanning dyes/stains other than Deep Purple, it is suggested that 1D or free floating gels are placed onto a clean glass plate for scanning purposes. This will reduce the possibility of cross contamination. If this method is used, the scanner 'Focal plane' setting may need to be set to +3mm instead of platen (if possible).

Cleaning and preparation of Bind-Silane coated plates

For complete removal of old gel and Bind-Silane:

- Thoroughly scrape off any residual bound gel with a plastic scraper.
- Wash the plates in freshly prepared 1%(v/v) Decon™ (branded Contrad™ in the USA) and wash with a soft sponge or brush to further remove the gel.
- Leave the plate to soak in 1%(v/v) Decon overnight.
- The following day, wash the plate with a soft sponge.
- Rinse the plate with water and leave the plate to soak in 1%(v/v) HCl for 1 hour.
- Wash the plate in 1%(v/v) Decon with a soft sponge or brush, then rinse with high purity water and leave to dry.
- Before using the plate, wipe over with ethanol, using a lint free cloth such as Crew Wipers.
- Bind-Silane should be applied to plates using a lint free cloth so that the solution is spread evenly. Long strokes, from one edge of the plate to the other, should be used, until the plate looks dry. The plate should then be covered with a dry cloth to prevent particulate contamination and left for at least 2 hours to dry completely.

Troubleshooting guide

Problems

1

Overall low signal intensity

Possible causes and solutions

Ensure that all steps in the protocol have been included and that incubation times have been followed correctly.

Ensure that the stain was not exposed to bright light either as the working stock solution or particularly during the staining process.

The use of too dilute or old ammonia development solution can lead to low signal intensity. Also ensure that the volume of development solution was correct for the volume of the gel.

Perform the 1:200 dilution of the Deep Purple stock solution only when required.

Use of too high concentration of ammonia or acid will cause degradation of fluorescent signal intensity and loss of sensitivity.

Re-use of the stain is not recommended.

Ensure Deep Purple dilution was 1:200 in water and that the correct volume was used for the volume of the gel (also taking into account the size of the container).

Check that the gel was incubated in the stain for at least the recommended period of time (staining for longer periods has not been seen to increase background).

2

Overall elevated background

Re-scan the gel at a higher PMT voltage (applicable to laser based fluorescent scanning instruments only).

Use a longer exposure time (for CCD based imaging instruments).

Fading of stain in a stored gel – ensure gels are stored in the recommended conditions and protected from light. Re-stain the gel if necessary.

Ensure correct fixation solution was used.

Prolong fixation to an overnight step.

Ensure ammonia development solution was at the correct concentration.

Perform extra washes with 0.75%(v/v) acetic acid after the development step.

Ensure correct volumes of wash solutions have been used for the volume of gel (also taking into account the size of the container).

Ensure that the platen of the imaging system is not contaminated with fluorescent compounds.

When using thicker gels or backed gels use an overnight fixation step.

Use a high quality SDS in the preparation and running of the gel (such as the USB product US75819); replace pre-made running buffers if necessary.

3**Localized patches of high background signal**

Platen of the imaging system may be contaminated with fluorescent compounds – follow the recommended cleaning procedure.

The SDS front on gels will be stained by Deep Purple and will appear as a dark band across the gel. Tracker dyes, such as bromophenol blue, can absorb fluorescent light resulting in a band that appears clearer than the background. To avoid this tracker dyes should be run off the bottom of the gel.

Handle gels with care as physical damage to gels may give a background stain in that area. Use clean powder free gloves.

If a dark stained area is seen at low mass / high pI on a 2D gel ensure that the fixation solution is as recommended and the fixation step is performed overnight.

4**Spots and streaks clearly visible in the background**

If using backed gels ensure that the gel plates are properly cleaned before use and that all gel material from previous experiments is completely removed.

Ensure that Bind-Silane coated plates are evenly coated, dried efficiently and free from particulate contamination.

Platen of the imaging system may be contaminated with fluorescent compounds

– follow the recommended cleaning procedure.

5

Small 'speckles' are seen on the image

Use clean powder free gloves.

Ensure gel containers are perfectly clean and rinsed before use. Ensure containers are free of contaminating fluorescent compounds.

6

Boundary or negative staining effects (bands / spots surrounded by a lighter stained area)

Inefficient removal of buffer components from the gel during fixation. Use methanol or ethanol in the fixation solution and perform fixation overnight.

Ensure that all wash steps in the protocol are performed as recommended.

Use a high quality SDS in the preparation and running of the gel (such as the USB product US75819); replace pre-made running buffers if necessary.

Quality control

A variety of QC tests are performed to check Deep Purple quality and performance. Each batch undergoes tests that include a measure of purity by HPLC and a test of performance in the fluorescence detection of proteins in gels.

Related products

Amersham Biosciences offers a comprehensive range of electrophoresis reagents and hardware all with proven compatibility to ensure reproducible high quality results. For a complete listing of products available see the current Amersham Biosciences catalogue or visit our web site at www.amersham.com.

References

1. Bell, P.J.L. and Karuso, P., *J. Am. Chem. Soc.*, **125**, 9304-5 (2003).
2. Mackintosh, J.A. *et.al.*, *Proteomics*, in press (2003).

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Product information

Product

Code

Deep Purple total protein stain

RPN6305

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Deep Purple total protein stain may only be used for applications in life science research.

“CyDye: this product or portions thereof is manufactured under license from Carnegie Mellon University under patent number 5268486 and other patents pending. Some of these products may only be available to collaborators and customers within certain of our technology access programmes. The purchase of CyDye DIGE Fluors includes a limited license to use the CyDye Fluors for internal research and development, but not for any commercial purposes. A license to use the CyDye Fluors for commercial purposes is subject to a separate license agreement with Amersham Biosciences”.

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