FT-1K9520

FluoProbes®

Protein Gel Stain 100X, RED Epicocconone based

Reversibly binds to proteins and peptides to yield an intensely red-fluorescent product for sensitive quantification of proteins in 1 & 2D gels of all chemistries, on both PVDF and nitrocellulose blots with compatibility with Mass Spectrometry

Product Description

Name:	Protein Gel Stain 100X, RED Epicoccone based
Catalog Number:	**1K952E**, 2mL (dilutes to 200mL) **1K9520**, 10mL (dilutes to 1L) **1K9521**, 50mL (dilutes to 5L)
Detection:	Excitation wavelengths: 405, 500nm. Suitable light sources include green (543, 532nm); blue (488nm); violet (405nm) or UVA. Emission wavelength: The maximum emission is at wavelength of 610nm regardless of what excitation source is used. Suitable filters include 610nm band pass or 560 long pass.
Storage:	Store at +4°C (up to 6 months) to protect from light. For long term storage, -20°C. Stable one month at room temperature.

Introduction

Peptide quantification is prerequisite in many areas of proteomics and peptidomics. Colorimetric assays (ninhydrin, Lowry, BCA) often lack the sensitivity to accurately quantify peptides. AAA is expensive, often inconvenient, and sensitive to many interfering compounds.

FluoProbes Peptide Quantification is a fluorescence-based peptide quantification kit. It works equally for protein[a] assay. FluoProbes Peptide Quantification is based on a small, naturally-occurring fluorescent compound[1] that reversibly binds to lysine, arginine, and histidine residues in peptides, and it responds to hydrophobic environments to yield an intensely red-fluorescent product[2]. This mechanism allows highly sensitive quantification of peptides over a wide linear dynamic range. Uniquely, FluoProbes Peptide Quantification tracelessly quantifies peptides, enabling the same sample to be used for peptide quantification and downstream analyses (e.g. MS, HPLC and Edman chemistry).

Quick Facts & Features

- Flexible: FluoProbes Total Protein Stain is suitable for staining proteins separated by either 1D or 2D electrophoresis on native or denaturing gels of all chemistries. FluoProbes Protein Gel Stain is also suitable for both PVDF and nitrocellulose blots.
- Compatible: Unique reversible staining by FluoProbes Protein Gel Stain makes it fully compatible with downstream processing (MS, immuno-staining and Edman sequencing). FluoProbes Protein Gel Stain shows higher MS compatibility than competing products .
- Environmentally friendly: FluoProbes Protein Gel Stain is based from an epicoccone core structure that is safe and simple to dispose.
- Health and safety: No requirement to store and handle volatile, corrosive acetic acid, heavy metal based or toxic stains.
- Simple and convenient: The protocol is simple (4 steps) and quick (3h).
- Sensitive: reliably provides <50pg sensitivity, in gels, when tested on 14 standard proteins with a range of molecular weights.
- Multiplex compatible: with other fluorophores (e.g. FluoProbes Dyes), other stains (e.g. Coomassie™).
- Clean background and no speckling: FluoProbes Protein Gel Stain does not produce speckles and has low background fluorescence.

Directions for use

Before use

Safe Handling and Disposal

All chemicals should be considered potentially hazardous. This product should only be handled by those persons trained in laboratory techniques, and used in accordance with the principles of good laboratory practice. Wear suitable protective clothing including laboratory overalls, safety glasses and gloves. FluoProbes Protein Gel Stain is a dilute solution of a natural organic dye in DMSO / acetonitrile. The diluted working solution is minimally hazardous and nonflammable; however the complete properties of the dye component have not been fully investigated.

Reagents and Equipment not provided

Solution 1 (fixation and acidification): Place 850mL of high purity water into a 1L bottle then add 10g citric acid (Cat. No. HO9790) and mix until dissolved. Add 150mL of 100% ethanol and mix thoroughly.

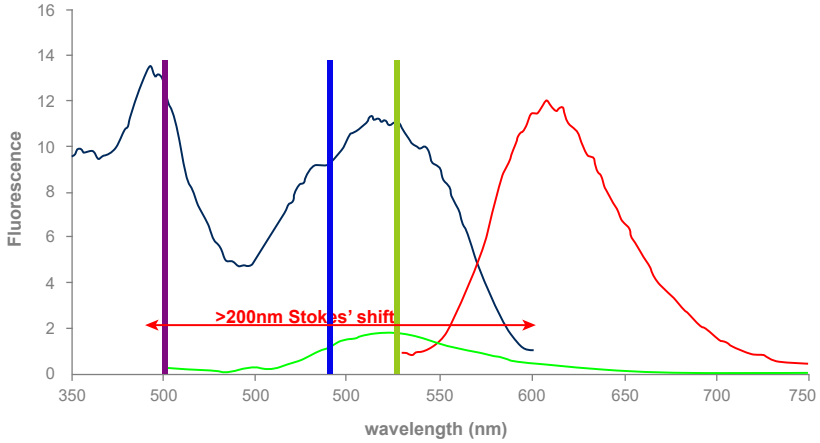
Please note: Due to volume contraction, the complete volume of this solution will be less than 1L.

Solution 2 (staining buffer): Add 1L of high purity water to a 1L bottle and add 6.2g boric acid (Cat. No. 07044B). Dissolve the powder by mixing using a magnetic stirrer (may take several minutes) then weigh and add 3.85g of sodium hydroxide (NaOH, p.a. quality) and continue stirring until fully dissolved. Alternatively, it is also possible to dissolve 9.5 g Borax ($Na_2B_4O_7 \cdot 10H_2O$) in 1L of high purity water. It is important that the pH of the final solution is > 9.4.
Solution 3 (washing): Mix 850mL of high purity water and 150mL of 100 % ethanol in a 1L bottle.
Please note: Due to volume contraction, the complete volume of this solution will be less than 1L.

Storage of solutions
Solutions 1-3 can be stored at room temperature and are stable for up to 6 months.
Solutions should be free of precipitation and protected from airborne particulate matter as this will result in speckling on the gels.

Detection
See the dye spectra.
Excitation: Optimum wavelengths at 405, 500nm.
Suitable light sources include green (e.g. 543, **532**nm) blue (e.g. **488**nm); violet (e.g. 405 nm) or UVA (**400**nm).
Emission: The maximum emission wavelength is at 610nm, irrespective of the excitation source. Suitable filters include the 610nm band pass or 560 long pass.

Fluorescence can be read by many platforms, such as fluorescence imager, fluorimeter, fluorescence plat readers, and laser scanner.



Staining of polyacrylamide gels
Fixation
• Fix gels in fixation solution 1 for a minimum of 1hr with gentle rocking.
• For appropriate volumes see table 1. For gels thicker than 1mm the fixation time should be extended to 1.5h.
The fixation time can be extended to overnight if required to decrease background or to fit into the workflow. Additional fixation washes may be used to decrease background levels even further, though this is generally not required.
• In parallel, if the FluoProbes Protein Gel Stain concentrate has been stored at -20°C, allow to warm to room temperature at this time.

Staining
Prepare the staining solution immediately prior to staining by thoroughly mixing the fully thawed concentrated stain and then diluting 1 part FluoProbes Protein Gel Stain concentrate in 100 parts staining buffer solution 2.
For appropriate volumes see *table 1*.

Please note: FluoProbes Protein Gel Stain slowly degrades over time in the high pH buffer.

If used immediately the staining solution may be re-used once for gel staining.
• Ensure the 1x staining solution is well mixed before adding to the gel.
• Remove the gels from the fixation solution 1 and place into the staining solution with gentle rocking.
• Stain for 1h for 1.0mm thick free-floating gels and for 1.5h for 1.5mm thick or 1mm thick film-backed gels.

IMPORTANT: Minimize carry-over of the acidic fixation solution (a short pre-buffering can increase staining effectivenes).

• Extending the staining time up to 2 h will not affect results.
• Do not stain for longer than 2 h.

Washing
• Remove the gels from the staining solution and wash the gels by gentle rocking in the washing solution 3 for 30min.
For appropriate volumes see *table 1*.
• For 1.5mm gels or if high background fluorescence is experienced washing time should be increased to 45min.

Acidification
• Remove the gels from the washing solution and acidify by placing them in solution 1 and rock gently for 30 min. For appropriate volumes see *table 1*.

This step may be repeated or extended up to overnight to reduce background staining). If left in the acidifier overnight, the gels should be protected from the light.

Table 1. Staining of polyacrylamide gels

Gel type		MiniGel	Standard Flatbed	Large Flatbed	Large gel (1mm thick)	
Process	Solution	Volume per gel				Time *
Fixing	Solution 1	100mL	200mL	300mL	400mL	1h
Staining	Solution 2	1mL dye in 100mL	2mL dye in 200mL	3mL dye in 300mL	4mL dye in 400mL	1h
Washing	Solution 3	100mL	200mL	300mL	400mL	30min
Acidification	Solution 1	100mL	200mL	300mL	400mL	30min

* For 1.5 mm or 1 mm backed gels extend time by 50 %.

IMPORTANT:

- The gel may be fixed in solution 1 overnight with no negative effects.
- Bring dye concentrate to room temperature and thoroughly mixed prior to being added.
- The staining solution must be made fresh (not more than 30 minutes prior to use).
- Add FluoProbes Protein Gel Stain concentrate to solution 2 before being poured onto the gel to prevent staining artifacts.
- It is not necessary to protect the gel from light.
- If there is no time for scanning you can leave the gel in the acidifier solution 1 overnight.

Storage

Gel should be stored at 4°C protected from light in 1 % (w/v) citric acid (storage solution). For extended storage (up to 6 months) add FluoProbes Protein Gel Stain (1:100) to the storage solution. Prior to imaging the gels should be rinsed (2 x 15min) in washing solution 3. Acidifying in solution 1 for 15min may be used to reduce background.

Staining of blotting membranes

For best results run the buffer front off the base of the gel during electrophoresis prior to transfer. Care should be taken to ensure that the membrane does not dry during the staining.

Care should be taken to ensure that the membrane does not dry during the staining.

Washing: Following transfer, place the wet membrane in water and wash for 3 x 5 min. For small-sized blots use 50mL for all steps, for large-sized blots use 200mL.

Basification: Wash the blot in solution 2 for 10 minutes.

Staining: For small blots add 625µL FluoProbes Protein Gel Stain to 50mL of high purity water.

For large blots add 2.5mL of FluoProbes Protein Gel Stain to 200mL of high purity water. Stain in FluoProbes Protein Gel Stain for 15-30min. Blots should be placed 'protein side' down in the prepared stain.

Follow either the PVDF or nitrocellulose protocol from this point.

PVDF membrane

Acidification: Place the blot in solution 1 and rock gently for 5min. For large blots use 400mL. For small blots use 50mL. Please note: This treatment will cause the blot to appear green.

Washing: Rinse blot with 100% methanol for 2-3min until green background on blot has been completely removed. Multiple rinses may be required.

Drying: Dry for 2-3min. To allow simultaneous drying of the blot on both sides it may be best to dry the blot on a wire mesh. Allow the blot to completely dry. The blot is then ready for imaging and further analysis.

Nitrocellulose

Washing: Place the blot in solution 2 and rock gently for 5min. Remove from solution 2 and place into high purity water and rock gently for 5min. Repeat the water washing.

Drying: Allow blot to completely dry. Your blot is now ready for imaging and further analysis.

Storage: PVDF and nitrocellulose blots should be stored dry, in the dark at room temperature.

De-staining

FluoProbes® Protein Gel Stain is reversible and the stain may be removed for subsequent analysis, for example by immuno staining. FluoProbes® Protein Gel Stain may be removed from blots without significant removal of proteins by washing membranes overnight in 50 mM ammonium carbonate solution.

For rapid **de-staining of PVDF membranes**, wash in 50% acetonitrile containing 30mM ammonium carbonate for 15min.

For rapid **de-staining of nitrocellulose membranes**, wash in 50% ethanol (methanol may be used) containing 50mM ammonium carbonate for 15min.

Note: The rapid de-staining protocols may result in loss of protein from the membrane.

Troubleshooting

Low signal intensity and poor sensitivity

• The most common cause of low signal intensity is poor basification.
Check the pH during the staining step; it should be between pH 9.5 and 10.5.
Carry-over of acid from the fixation step is a common cause of poor basification and thus staining.

Too acidic conditions turn the color of the staining solution into yellow!

It is helpful to apply a short pre-buffering step with Solution 2 or used staining solution prior to staining.
• Ensure you use the appropriate volume of solutions, smaller volumes can reduce staining effectiveness.
• Ensure that you use the stain at the recommended 1:100 dilution.
Greater dilution will result in lower fluorescence intensity.
• Long exposure time and associated heating on CCD-based instruments may cause the stain to fade.
• Ensure you are using the correct filters, photomultiplier tube settings and light source on your scanner.
• Ensure the concentrated FluoProbes Protein Gel Stain was brought to room temperature and thouroghly mixed prior to dilution to 1x.
• Ensure that the correct fixation solution was used.

High Background

• The staining tray shouldn't be used for Coomassie staining before.
• Ensure that the stain was fully mixed into the borate buffer before adding to the gel.
• Ensure correct volumes for gel solutions have been used.
• Ensure only one gel per tray is stained. Multiple gels in one tray can result in an uneven background.
• Ensure gels are not handled or only with clean gloves to avoid contamination with dust and/or protein.
• For thicker (>1mm) or backed gels you may need to extend fixing, washing and acidification times.
• Ensure the concentrated FluoProbes Protein Gel Stain was brought to room temperature and thoroughly mixed prior to dilution to 1x

Boundary or negative staining

• Ensure you use a high quality SDS in the preparation and running of the gel.
• Extend the fixation time to overnight.
• Ensure you use sufficient fixation and washing solutions.
• Extend your washing time.

References

- **Peixoto P.** et al., Design and Synthesis of Epicocconone Analogues with Improved Fluorescence Properties, JACS, 136, 15248–15256 (2014)

Related Products

• Citric acid, HO9790
• Boric acid, 07044B
• Acrylamide:bis-acrylamide 37,5:1, UP864937
• 3Dye Cy2/3/5 fluor Labeling Pack, EV0870

Other Information

For any question, please ask FluoProbes®: Hotline: +33 4 70 03 73 06
Catalog size quantities and prices may be found at http://www.interchim.com.

Legal

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