CYANAGEN

*Reagents for Molecular Biology*

NEW

THE RIGHT LIGHT

# RUBY GEL STAIN

Fluorescent stain for
protein detection in gel

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# About us

Cyanagen is a biotech company located in Bologna, dedicated to research, development and production of reagents for molecular diagnostic since 2003 and one of the leading companies in the field of reagents for Western blotting and Elisa.
The main product lines are focused on chemiluminescence and fluorescent dyes for biological analysis, genomics, proteomics and chemical sensors.
They are based on Cyanagen internationally patented technologies and achieve outstanding performance in terms of sensitivity and stability.
The products are extremely versatile and perfectly suited to the latest analytical instrumentation. These products are also available as OEM.

Cyanagen s.r.l. has a certified Quality System

ISO 9001-2008 QUALITY CERTIFIED

# Product manual

# Ruby Gel Stain

Fluorescent stain for protein detection in gel

**RUBY PROTEIN STAIN IS INTENDED FOR RESEARCH USE ONLY AND SHALL NOT BE USED IN ANY CLINICAL PROCEDURES OR FOR DIAGNOSTIC PURPOSES.**

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# 1. Introduction

Ruby Gel Stain is a ready to use kit for rapid and sensitive protein staining in 1D and 2D SDS polyacrylamide electrophoresis gels. It enables optimal visualization and quantitation of proteins with high contrast and the same sensitivity of silver staining without its drawbacks. The staining procedure is a simple 220 minutes three steps protocol.

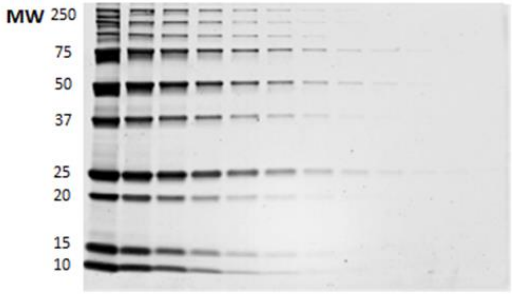
After staining, proteins can be removed from the gel and analyzed by mass spectrometry without interference from stain.

The dye has optimal excitation at 302 and 470 nm with an emission maximum at approximately 610 nm.

Ruby Gel Stain can be excited with UV-light, UV transilluminator and 405, 445, 473-488 nm laser sources.

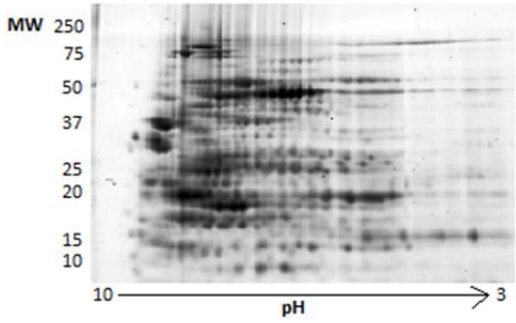
**Ruby Gel Stain**

1D gel



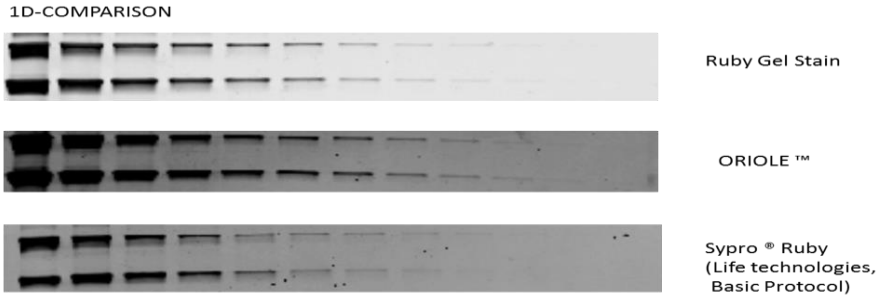

**a**

2D gel




**b**

**a)** Ruby Gel Stain on a 1D gel. Two-fold dilution series of Precision Plus Protein™ Unstained BIO-RAD marker separated on a BIO-RAD Mini-PROTEAN™ TGX™ 12% and stained following Ruby Gel Stain 220' min protocol. Images acquired by GEHC ImageQuant™ LAS4000, Exc 312 nm, exposure time 1s. Lane 9 is approximately 0.4 ng **b)** Ruby Gel Stain on a 2D gel. 60 µg of Bio-Rad ReadyPrep™E.coli Protein Sample, separated on Bio-Rad ReadyPrep™ IPG strips pH 3-10 NL and then on a SDS-PAGE (T%8-16) with a Bio-Rad PROTEAN™ II XI system. The gel was stained following Ruby Gel Stain 220' min protocol.
The image was digitalized by Bio-Rad Molecular Imager FX™Pro fluorescent imager, PMT Voltage Medium Sample Intensity.



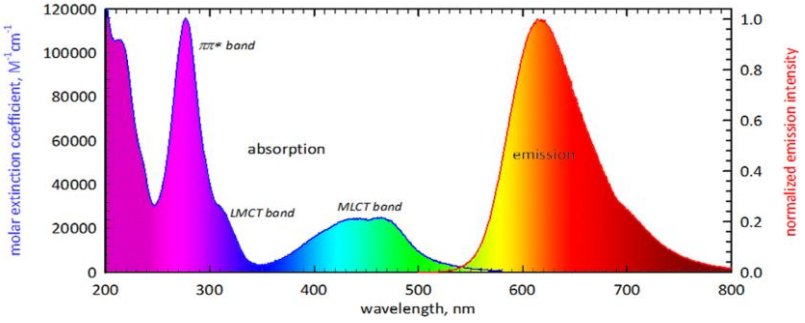

Ruby Gel Stain on a 1D gel. Two-fold dilution series of Precision Plus Protein™ Unstained Bio-Rad marker, separated on a Bio-Rad a Mini-PROTEAN® TGX™ 12%.

Lane 9 is approximately 0.4 ng.

**Advantages:**

- High purity dye: >98%;
- Optimal signal to background ratio;
- Strong, uniform and reproducible signals from 0.2 ng to 10 ng protein;
- Fast staining protocol (220 min).
- Convenient: fixing and destaining solutions included in the kit.

The gel can be visualized using excitation wavelength in the 400/500 nm range with bright emission peak centred around 610 nm.




Absorption and emission spectra of Ruby Protein Stain

## Storage

- Stable at room temperature;
- Avoid prolonged exposure to temperatures greater than 37°C and protect from light.

# 2. Important notes

The dye is deeply colored, care and use of gloves and suitable protective clothing to handle the vials is recommended.

# 3. Components and other materials required

**PRTD066,0050 (Solution A 50 ml, Solution B 100 ml) enough stain for:**

- 1 minigel (8.6 x 6.7 x 0.1 cm)

**PRTD066,0250 (Solution A 250 ml, Solution B 500 ml) enough stain for:**

- 5minigels (8.6 x 6.7 x 0.1 cm)
- 2 medium format gels(13.3 x 8.7 x 0.1 cm)
- 1 large format gel (16 x 20 x 0.1 cm)

# 4. Staining protocol (total 220 min):

1. **Fix (60 minutes):**
   - Remove the gel from the gel cassette or plates.
   - Place it in a clean plastic container.
   - Incubate gel in the **Ruby Gel Stain Solution B** for 1 hour using a rocker. The volumes used for typical gels are given in the listed below.

| Gel size | Required volume of solution B |
|---|---|
| Minigel (8.6 x 6.7 x 0.1 cm) | 50 ml |
| Medium format gel (13.3 x 8.7 x 0.1 cm) | 100 ml |
| Large format gel(16 x 20 x 0.1 cm) | 250 ml |

2. **Stain (60 minutes):**
   - Transfer the gel to a clean container and incubate it in the **Ruby Gel Stain Solution A** for 1 hour using a rocker. Wrap the staining container in aluminum foil to protect from direct light. **Approximately 10 times the volume of a given gel is needed for efficient staining.** The volumes used for typical gels are listed in the table below.

| Gel size | Required volume of solution A |
|---|---|
| Minigel (8.6 x 6.7 x 0.1 cm) | 50 ml |
| Medium format gel (13.3 x 8.7 x 0.1 cm) | 100 ml |
| Large format gel(16 x 20 x 0.1 cm) | 250 ml |

3. **Destain (80 minutes):**
    - Transfer the gel to a clean container to minimize background staining irregularities and stain speckles.
    - Destain the gel in **Ruby Gel Stain Solution B**. Wrap the container in aluminum foil to protect from direct light. The volumes used for typical gels are given in the table above.The volumes used for typical gels are listed in the table below.

| Gel size | Required volume of solution B |
|---|---|
| Minigel (8.6 x 6.7 x 0.1 cm) | 50 ml |
| Medium format gel (13.3 x 8.7 x 0.1 cm) | 100 ml |
| Large format gel(16 x 20 x 0.1 cm) | 250 ml |

4. **Wash (20 minutes):**
    - Transfer the gel to a clean container and equilibrate it for 20 minutes in deionized water.
5. **Scan:**
    - Clean the surface of the transilluminator or sample tray, prior to and after each use, using deionized water.
    - The dye has optimal excitation at 302 and 470 nm with an emission maximum at approximately 610 nm.
    - Ruby Gel Stain can be excited with UV-light, UV transilluminator and 405, 445, 473-488 nm laser sources.

**Important note:**

- Always wrap the staining container in aluminum foil to protect from direct light;
- Perform all steps with continuous and gentle agitation using a rocker;
- Staining containers should be meticulously clean, we recommend polypropylene or polycarbonate container to avoid the loss of dye;
- Wear clean powder free gloves during all gel handling.

# 5. Troubleshooting

| Problem: | Possible Cause: | Suggestion: |
|---|---|---|
| Gels tearing and breaking during fix/stain/destain | Shaking motion too vigorous; sharp utensils used to handle gel. Wear and tear on the gel is also proportional to the number of solution changes, the total time of the staining procedure, and the amount the gel is handled. | Decrease shaker speed/motion, use smooth plastic utensils. |
| Elevated background levels | Some gel types, such as gradient gels, tend to show increasing background levels toward the bottom of the gel; very thick gels have higher background staining. | Increase the destain step for further 15/30 minutes. |
| Spots and streaks are visible, or there is an uneven background | Contamination of solutions used to make in-house poured gels, running buffer or sample loading buffer; poor water quality; contamination of imager surface with fluorescent compounds; handling of gel with bare hands or powdered gloves; staining of gel with insufficient agitation; incomplete immersion of gel during staining. | Use freshly made and filtered solutions, or buy premade gels; use ultrapure water of >18 megohm-cm resistance; use glass columns and sterile pipettes to prepare reagents; wash glassware thoroughly; clean the surface of the imaging system thoroughly with 100% ethanol followed by ultrapure water; always handle gels with powder-free gloves; perform all incubations for staining and washing steps on an orbital shaker set at 50–60 rpm. |
| Small speckles are seen in the image | Poor water quality; contamination by lint or dust on the surface of the gel or imager; Ruby Gel Stain that has accumulated on the surface of the staining container during long incubations has come off during the wash step. | Use ultrapure water of >18 megohm-cm resistance; use lint-free wipes; increase destain step (15/30 minutes more), which minimizes the time available for dye aggregate formation; transfer the gel to a clean |

| | | |
|---|---|---|
| | | container between the stain and the wash step. |
| Presence of a 50–68 kDa band in all lanes | Contamination with keratin from skin or hair. | Use clean gloves when handling and loading gels. Rinse all wells of the gel with ultrapure water before sample loading. |

# 6. Ordering information

| Product Description: | Quantity: | Sufficient For: | Order-No: |
|---|---|---|---|
| Ruby Gel Stain | Solution A 50 ml<br>Solution B 100 ml | 1 Mini-PROTEAN®gels<br>(8.6 x 6.7 x 0.1 cm) | PRTD066,0050 |
| | Solution A 250 ml<br>Solution B 500 ml | 5 Mini-PROTEAN® gels<br>(8.6 x 6.7 x 0.1 cm)<br>2 Criterion® gels<br>(13.3 x 8.7 x 0.1 cm)<br>1 PROTEAN® II gels<br>(16 x 20 x 0.1 cm) | PRTD066,0250 |

For further information, visit **www.cyanagen.com**

For orders: call **+39 051.534063**
mail to **sales@cyanagen.com**

CYANAGEN

*Reagents for Molecular Biology*

Cyanagen s.r.l.
Via Stradelli Guelfi, 40/C
40138 Bologna (ITALY)

T +39 051 534063
info@cyanagen.com
www.cyanagen.com

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ISO 9001
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