## INSTRUCTION MANUAL

# AquiStain RED

Fluorescent Total Protein Stain **100X**,

for Polyacrylamide Gels and Blotting Membranes

AQUISTAIN

ECO-FRIENDLY PROTEIN STAINING

Version 2 – 14/12/2016

**AquiStain** – Bâtiment A11, 351 Cours de la Libération, 33400 Talence **– FRANCE**

+33 (0)6 33 63 66 53 – contact@aquistain.com – http://www.aquistain.com

## IMPORTANT INFORMATION

The following instructions are for use with **AquiStain RED** Total Protein Stain.

## STORAGE INFORMATION

Store **AquiStain RED Dye** in a fridge at + 4°C (up to 6 months) in the original brown bottle provided and protect from light. For long term storage, store the dye in a freezer at – 15°C to – 30°C.

## WARNINGS AND PRECAUTIONS

- **AquiStain RED** Total Protein Stain is for research use only
- Always wear gloves when handling membranes and reagents
- Refer to MSDS for additional safety information
- The product is guaranteed to be free of manufacturer defect, and to function as described when the enclosed protocol is followed by properly trained personnel.

## 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. **AquiStain RED** is a dilute solution of a synthetic organic dye in DMSO. The diluted working solution is minimally hazardous and non-flammable; however the complete properties of the dye component have not been fully investigated.

# TABLE OF CONTENTS

# 1. KIT CONTENTS

**AquiStain RED** Total Protein Stain, **25 mL**, is sufficient for staining **fifty** SDS-PAGE mini-gels (8 cm × 11 cm) or **ten** full-size 2D gels (17 cm × 17 cm).

**AquiStain RED** Total Protein Stain is also available in 5 mL and 100 mL packs.

# 2. SHIPPING AND STORAGE CONDITIONS

Shipping at ambient temperature (below 25°C) is acceptable if the total dispatch time is no longer than 5 days. Upon receipt, store **AquiStain RED Dye** in a fridge at + 4°C (up to 6 months) in the original brown bottle provided and protect from light. For long term storage, store the dye in a freezer at – 15°C to – 30°C.

# 3. ADDITIONAL MATERIALS REQUIRED

- High-purity water (distilled, Milli Q or equivalent)
- 100% ethanol
- Citric acid
- Boric acid or Borax
- NaOH (p.a. quality)
- Staining tray (Do not use metal trays, you can use dark or transparent plastic trays).
- Shaking or rocking platform
- Imaging systems

# 4. About AquiStain RED

**AquiStain RED** is an eco-friendly fluorescent dye that covalently and reversibly binds to lysine, arginine and histidine residues in proteins and peptides to yield an intensely red-fluorescent product. This unique mechanism provides sensitive quantification of proteins in 1D and 2D gels of all chemistries, on both PVDF and nitrocellulose blots and provides unparalleled compatibility with Mass Spectrometry.

# 5. Excitation and Emission Spectra

Optimal excitation wavelengths for **AquiStain RED** are 518 nm. Compatible excitation light sources include green (532 nm), blue (488 nm), violet (405 nm) or UV (365 nm). The maximum emission wavelength of **AquiStain RED dye** is 610 nm regardless of what excitation source is used. 610 nm band pass or 560 long pass filters may be used. The excitation and emission spectra of **AquiStain RED** can be seen in Figure 1.

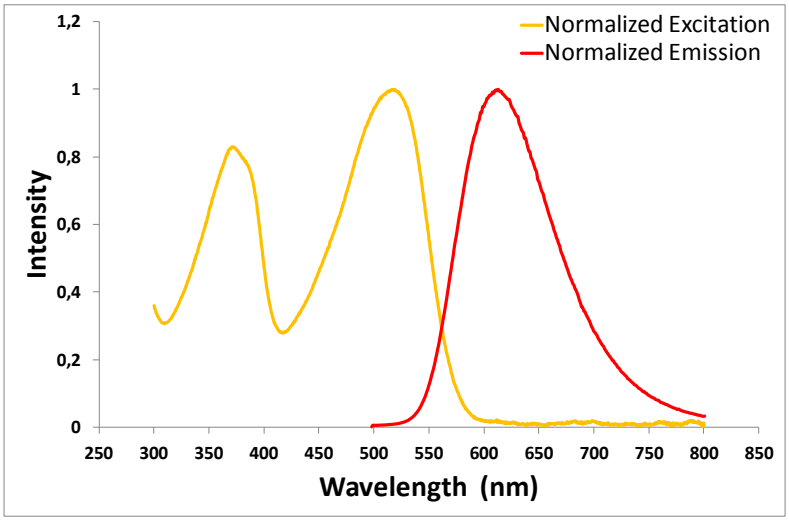


**Figure 1.** Excitation and Emission Spectra of **AquiStain RED** Dye with BSA

# 6. Overview of AquiStain RED Gel Staining Protocol

**Fixation**

Incubate gel in Fix Solution 1 hour to overnight

↓

**Pre - buffering**

Rinse gel with high-purity water and incubate in Stain Buffer 5 minutes

↓

**Staining**

Incubate gel in Stain Solution 1 to 2 hours

↓

**Washing**

Rinse gel with high-purity water and incubate in Wash Solution 30 to 45 minutes

↓

**Acidification**

Incubate gel in Acid Solution 30 minutes to overnight

↓

**Image**

# 7. Preparation of Solutions

Before staining, prepare Fix, Acid, Stain, and Wash solutions as described below. These solutions are stable for up to 1 year when stored at room temperature. Precipitates or dust present in the solutions will result in speckling on gels. If observed, filter solutions before use.

**Fix and Acid Solutions**

Add 10.1 g of citric acid to 850 mL of high-purity water in a 1L bottle. Mix until dissolved. Add 150 mL of 100% ethanol and mix thoroughly.

The pH of the Fix Solution should be approximately pH = 2.3.

**Stain buffer** (100 mM Sodium Borate)

Add 6.2 g of boric acid to 1L of high-purity water in a 1L bottle. Dissolve the powder by mixing using a magnetic stirrer (may take several minutes) then add 3.85 g of sodium hydroxide (NaOH, p.a. quality). Mix until completely dissolved.

Alternatively, it is also possible to dissolve 9.5 g of Borax ($Na_2B_4O_7.10H_2O$) in 1L of high-purity water.

It is important that the pH of the final solution is approximately pH = 10.5.

**Wash Solution**

Mix 850 mL high-purity water and 150 mL 100% ethanol in a 1L bottle.

# 8. Detailed Protocol, Gel Staining

| Step | Notes |
|---|---|
| **1. Fixation** | |
| • Fix gel in Fix Solution for a minimum of 1 hr with gentle rocking.<br>• For correct volumes at each step, refer Table 1.<br>• In parallel, if the **AquiStain RED Dye** has been stored at – 20°C, allow to warm to room temperature at this time. | • For gels thicker than 1 mm or backed gels, increase the fixation time to 1.5 hr.<br>• The fixation time can be extended to overnight. |
| **2. Pre - Buffering** | |
| • Remove gel from Fix Solution, rinse with high-purity water, and incubate in Stain Buffer for 5 min. | • For small blots, use 50 mL.<br>• For large blots, use 250 mL. |
| **3. Staining** | |
| • Prepare the Stain Solution immediately prior staining.<br>• Remove gel from Stain Buffer and place in Stain Solution.<br>• Stain gel for 1 hr with gentle rocking. | • **To prepare Stain Solution**: Allow **AquiStain RED Dye** to warm to room temperature. Mix thoroughly, then dilute 1 part **AquiStain RED Dye** in 100 parts Stain Buffer. Mix well. Refer to Table 1 for volumes of solutions used for different gel sizes.<br>• Stain Solution will degrade over time. Prepare only as much as is needed and use immediately.<br>• Increase staining time to 1.5 hr for gels 1.5 mm thick or backed gels. Extending the staining time to 2 hours will not affect results.<br>• **DO NOT** stain for longer than 2 hrs. |
| **4. Washing** | |
| • Remove gel from Stain Solution, rinse with high-purity water, and wash in Wash Solution for 30 min with gentle rocking. | • For 1.5 mm thick gels, or gels with high background fluorescence, increase washing time to 45 min. |
| **5. Acidification** | |
| • Remove gel from Wash Solution and place in Acid Solution.<br>• Incubate in Acid Solution for 30 min with gentle rocking. | • This step can be repeated or extended to overnight to reduce background staining.<br>• If performing this step overnight, protect the gel from light. |
| **6. Imaging** | |
| • Detect fluorescence at 610 nm using standard fluorescence scanners and CCD camera systems. For recommended imaging setting, refer to Table 3. | • Compatible excitation sources include green (532 nm), blue (488 nm), violet (405 nm) or UV (365 nm).<br>• Detect fluorescence using 610 nm band pass or 560 nm long pass filter. |

| Gel Dimensions | Solution | | | | |
|---|---|---|---|---|---|
| | Fix | Stain | | Wash | Acid |
| | | Stain Buffer | AquiStain RED Dye | | |
| 8 cm × 11 cm × 1 mm (**mini-gels**) | 100 mL | 50 mL | 500 µL | 100 mL | 100 mL |
| 13.3 cm × 8.7 cm × 1 mm (small format 2D gels) | 200 mL | 100 mL | 1 mL | 200 mL | 200 mL |
| 17 cm × 17 cm × 1 mm | 500 mL | 250 mL | 2.5 mL | 500 mL | 500 mL |
| 17 cm × 17 cm × 1.5 mm | 500 mL | 250 mL | 2.5 mL | 500 mL | 500 mL |
| 15 cm × 19 cm × 1 mm | 500 mL | 250 mL | 2.5 mL | 500 mL | 500 mL |
| 15 cm × 19 cm × 1.5 mm | 500 mL | 250 mL | 2.5 mL | 500 mL | 500 mL |
| 20 cm × 25 cm × 1 mm | 750 mL | 375 mL | 3.75 mL | 750 mL | 750 mL |
| 20 cm × 25 cm × 1.5 mm | 750 mL | 375 mL | 3.75 mL | 750 mL | 750 mL |

**TABLE 1. VOLUMES OF SOLUTIONS FOR DIFFERENT GEL SIZES**

| Imaging System | Excitation | Emission | Notes |
|---|---|---|---|
| Laser scanner | Green (532 nm) light | Orange long pass (560 nm) filter or red band pass (610 nm) filter | |
| CCD imager with transilluminator | Long wavelength UV (302/365 nm) or black light blue lamp | Orange long pass (560 nm) filter | |
| Ettan™ DIGE Imager (GE Healthcare) | green (540/25 nm) light source | Orange (595/25 nm) filter | For multiplex applications, violet excitation filter (390/20 nm) and orange emission filter will avoid cross talk with Cy2 and Cy3. |

**TABLE 2. RECOMMANDED IMAGING CONDITIONS FOR DIFFERENT IMAGING SYSTEMS**

# 9. Detailed Protocol, PVDF Blot Staining

| Step | Notes |
|---|---|
| **1. Washing** | |
| • Following transfer, wash blot 3 times in high-purity water for 5 min with gentle rocking. | • For best results, run the buffer front off the base of the gel during electrophoresis prior to transfer.<br>• Do not allow membrane to dry during staining. |
| **2. Basification** | |
| • Remove gel from high-purity water, and incubate in Stain Buffer for 10 min. | • For small blots, use 50 mL.<br>• For large blots, use 400 mL. |
| **3. Staining** | |
| • Prepare the Stain Solution immediately prior staining.<br>• Remove gel from Stain Buffer and place in Stain Solution.<br>• Place blot protein side down in Stain Solution.<br>• Stain blot with gentle rocking for 15 – 30 min. | • **Prepare Stain Solution:** allow **AquiStain RED Dye** to warm to room temperature. Mix thoroughly.<br>• For small blots, dilute 250 µL of **AquiStain RED Dye** in 50 mL Stain Buffer, Mix well.<br>• For large blots, dilute 2 mL of **AquiStain RED Dye** in 400 mL Stain Buffer. Mix well. |
| **4. Acidification** | |
| • Place blot in Acid Solution and incubate with gentle rocking for 5 min. | • Blot will appear green.<br>• For small blots, use 50 mL.<br>• For large blots, use 400 mL. |
| **5. Wash** | |
| • Rinse blot 3 times with 100% ethanol for 2-3 min each, until green background on blot has been completely removed. | • Methanol may used instead of ethanol. |
| **6. Drying** | |
| • Hang blot from a peg or dry on wire mesh to allow blot to dry evenly.<br>• Allow blot to dry completely before imaging. | |

# 10. Detailed Protocol, Nitrocellulose Blot Staining

| Step | Notes |
|---|---|
| **1. Washing** | |
| • Following transfer, wash blot 3 times in high-purity water for 5 min with gentle rocking. | • For best results, run the buffer front off the base of the gel during electrophoresis prior to transfer.<br>• Do not allow membrane to dry during staining. |
| **2. Basification** | |
| • Remove gel from high-purity water, and incubate in Stain Buffer for 10 min. | • For small blots, use 50 mL.<br>• For large blots, use 400 mL. |
| **3. Staining** | |
| • Prepare the Stain Solution immediately prior staining.<br>• Remove gel from Stain Buffer and place in Stain Solution.<br>• Place blot protein side down in Stain Solution.<br>• Stain blot with gentle rocking for 15 – 30 min. | • **Prepare Stain Solution**: allow **AquiStain RED Dye** to warm to room temperature. Mix thoroughly.<br>• For small blots, dilute 250 µL of **AquiStain RED Dye** in 50 mL Stain Buffer, Mix well.<br>• For large blots, dilute 2 mL of **AquiStain RED Dye** in 400 mL Stain Buffer. Mix well. |
| **4. Acidification** | |
| • Place blot in Acid Solution and incubate with gentle rocking for 5 min. | • Blot will appear green.<br>• For small blots, use 50 mL.<br>• For large blots, use 400 mL. |
| **5. Wash** | |
| • Wash blot 1 time in Stain Buffer for 5 min with gentle rocking.<br>• Remove gel from Stain Buffer and place in high-purity water.<br>• Wash blot 2 times in high-purity water for 5 min with gentle rocking. | |
| **6. Drying** | |
| • Allow blot to dry completely before imaging. | |

## 11. DE-STAINING

**AquiStain RED** staining is reversible and the stain may be removed for subsequent analysis such as Western blotting.

- **To de-stain while minimizing protein loss:**
  Wash gel or blot overnight in 50 mM ammonium carbonate solution.

- **To rapidly de-stain PVDF membranes:**
  Wash blot with 50% acetonitrile containing 30 mM ammonium carbonate for 15 min.

- **To rapidly de-stain nitrocellulose membranes:**
  Wash blot with 50% ethanol or methanol containing 50 mM ammonium carbonate for 15 min.

- **To rapidly de-stain protein gels:**
  Wash gel with 50% ethanol or methanol containing 50 mM ammonium carbonate for 15 min to 1 hour.

## 12. STORAGE

**Gels** may be stored at 4°C in 1% (w/v) citric acid (storage solution) and protected from light. For extended storage (up to 6 months), add **AquiStain RED Dye** to the storage solution at 1:100. Prior to imaging, rinse gels 2 × 15 min in Wash Solution. Incubating in Acid Solution for 15 min can reduce background.

**Blots** may be stored dry, in the dark, at room temperature.

## 13. OPTIMIZATION

For a better fixation of proteins on the gel and to prevent the diffusion of proteins in the matrix, ethanol and citric acid of the fix solution can be replaced by methanol (40% - v/v) and acetic acid (10% - v/v), respectively.

Mix 500 mL high-purity water, 400 mL 100% methanol and 100 mL glacial acetic acid in a 1L bottle. This solution is stable for up to 1 month at room temperature.

Fix gel in this Fix Solution for **30 min** with gentle rocking.

# 14. TROUBLESHOOTING & FAQ

| Problem | Possible Solutions |
|---|---|
| **High Background** | • Handle gels with clean non-powdered gloves, and avoid contamination with dust and/or protein.<br>• Ensure concentrated **AquiStain RED Dye** was brought to room temperature and thoroughly mixed prior dilution.<br>• Ensure stain was thoroughly mixed into Buffer Stain before adding to gel.<br>• Stain only one gel per tray.<br>• Use high-purity water (distilled, Millli-Q, or equivalent).<br>• The staining tray shouldn't be used for Coomassie staining before. |
| **No or low signal** | • Check pH during staining step; pH should be between 9.5 and 10.5. Carry-over acid from the fixation step can result in poor staining.<br>• Stain may fade with long exposure times and associated heating on CCD-based instruments.<br>• Ensure stain concentrate was brought to room temperature and mixed thoroughly before dilution.<br>• Staining for over 2 hours in basic conditions destabilizes proteins, and leads to diffusion of protein bands from the gel matrix. |
| **Negative staining** | • Use high-quality SDS in the preparation and running of the gel.<br>• Extend fixation time to overnight.<br>• Use correct volumes of Fix, Wash, and Acid Solutions.<br>• Extend washing time. |
| **Speckled background** | • Filter buffers to remove dust or precipitates.<br>• Protect gel from airborne particles. |

# 15. REFERENCES

- Peixoto, P.A.; Boulangé, A.; Ball, M.; Naudin, B.; Alle, T.; Cosette, P.; Karuso, P.; Franck, X. *J. Am. Chem. Soc.* **2014**, *136*, 15248-15256.

- Moritz, C.P.; Marz, S.X.; Reiss, R.; Schulenborg, T.; Friauf, E. *Proteomics* **2014**, *14*, 162-168.