ProStain™
Protein Quantification Kit

(version D2)

Catalog No. 15001

TABLE OF CONTENTS Page

Introduction

Active Motif's ProStain™ Protein Quantification Kit simplifies protein quantification by providing highly sensitive detection reagents in a convenient, easy-to-use format. The kit's detection reagent is also resistant to many effects, which can limit the usefulness of other protein quantification systems, such as pH and many commonly found contaminants; for example, detergents and salts. In addition, the large Stokes shift, fast reaction time and limited free dye quantum yield make this kit a significant improvement over other photometric or fluorescent-based detection systems.

The kit provides lyophilized dye reagent, dilution buffer and BSA for preparation of standards. Simply resuspend the lyophilized dye in methanol to create the concentrated stock solution, dilute the stock solution, load 100 µl into the wells of a microplate, add 100 µl of sample, mix, then read the fluorescence. The assay is performed at room temperature, and the signal is stable for up to 2 hours. Common contaminants, such as pH, salts, solvents and some detergents are well tolerated in this assay, but buffers containing high amounts of free amines will affect sensitivity.

Advantages

- Large Stokes shift for reduced background
- Fast and simple procedure
- Robust – limited effect from contaminating substances
- Increased quantum yield for improved sensitivity and wide dynamic range

product	format	catalog no.
ProStain™ Protein Quantification Kit	1000 assays	15001

* Sufficient components are provided for performing 1000 assays using fluorescent-based detection. This assay can also be easily adapted for use in smaller or larger formats such as 384-well plates or cuvettes.

ProStain™ is for research use only. Not for use in diagnostic procedures.

Kit Components and Storage

The ProStain Kit is for research use only. Not for use in diagnostic procedures. Kit components arrive at room temperature. We recommend storing each component at the temperatures recommended in the table below:

Reagents	Quantity	Storage / Stability
Dye Reagent AM1	0.5 mg	4°C for 6 months
Dilution Buffer	100 ml	4°C for 6 months
BSA	1 mg	4°C for 6 months

Additional materials required

- Multi-channel pipettor & pipettor reservoirs
- Fluorescent detector
- Distilled water
- Black microtiter plates or cuvettes
- Methanol (for reconstituting the Dye Reagent)

Preparation of Reagents

Dye Reagent Stock Solution

The Dye Reagent is supplied lyophilized. Prepare the Dye Reagent Stock Solution by resuspending the lyophilized Dye Reagent in 12.5 ml methanol in the provided amber bottle. This stock solution can be stored at 4°C for 6 months.

Dye Reagent Working Solution

Prepare the Dye Reagent Working Solution by diluting the Dye Reagent Stock Solution 1:10 with distilled water. This solution should be prepared fresh on the day the assay is performed.

Reagent	10 rxns	50 rxns	100 rxns
Dye Reagent Stock Solution	110 µl	550 µl	1.1 ml
Distilled H_2O	990 µl	4.95 ml	9.9 ml

Dilution Buffer

This is supplied ready to use.

Stock BSA Solution

The BSA is supplied lyophilized. Prepare the Stock BSA Solution by resuspending the lyophilized BSA in 1 ml of dH_2O in the provided tube to make a 1 mg/ml solution. This stock solution can be stored at -20°C for 6 months.

PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING!

1. Remove kit contents from 4°C and bring all components to room temperature before use.
2. Set up a BSA Standard Curve in duplicate using the following concentrations: 10.0, 5.0, 2.5, 1.25, 0.63, 0.32, 0.15 and 0.0 µg/ml. (See table below for suggested layout.)
3. Add 198 µl of Dilution Buffer to wells A1 and A2.
4. Add 100 µl of Dilution Buffer to wells B1 through H1 and B2 through H2
5. Pipette 2 µl stock BSA solution (1 mg/ml) into wells A1 and A2.
6. Mix wells A1 and A2 by pipetting.
7. Transfer 100 µl from well A1 to B1 and A2 to B2.
8. Mix wells B1 and B2 by pipetting.
9. Transfer 100 µl from well B1 to C1 and B2 to C2.
10. Continue this procedure to wells G1 and G2. After mixing, discard 100 µl of solution from wells G1 and G2.
11. Wells H1 and H2 are blanks and should contain only 100 µl of Dilution Buffer.

	1	2	3	4	5	6	7	8
A	10.0 µg/ml	10.0 µg/ml	–	–	–	–	–	–
B	5.0 µg/ml	5.0 µg/ml	–	–	–	–	–	–
C	2.5 µg/ml	2.5 µg/ml	–	–	–	–	–	–
D	1.25 µg/ml	1.25 µg/ml	–	–	–	–	–	–
E	0.63 µg/ml	0.63 µg/ml	–	–	–	–	–	–
F	0.32 µg/ml	0.32 µg/ml	–	–	–	–	–	–
G	0. 15 µg/ml	0. 15 µg/ml	–	–	–	–	–	–
H	Blank	Blank	–	–	–	–	–	–

12. **Sample wells:** For protein determination of unknown samples, prepare a series of dilutions with the Dilution Buffer, for example: 1:50, 1:100 and 1:200. Pipette 100 µl into each well. Duplicates of each sample are recommended.

13. Add 100 µl/well of Dye Reagent Working Solution (see Preparation of Reagents section on page 2 for directions on preparing the Dye Reagent Working Solution) and mix by pipetting up and down.

14. Incubate for 30 minutes at room temperature (20-25°C) without agitation.

    Note: The signal intensity is stable for up to 1.5 hours after the 30-minute incubation; read sample before 1.5 hours have elapsed.

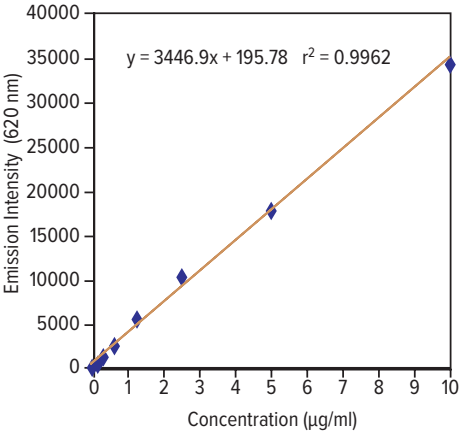
15. Measure the fluorescence (excitation: 488 nm, emission: 635 nm).

    Note: When measuring fluorescence, ensure that the gain settings are set to optimal (~ 140 gain), the number of flashes are set to 3 with no lag time, and the integration time is ~ 40 µs.

16. Use the standard curve to calculate the protein concentrations of the unknown samples. For the BSA standard curve, fit with the function y= Ax + B. The r^2 should be over 0.95.

Calculation of protein concentration using the BSA standard curve

Average the duplicate readings for each standard and sample and subtract the value obtained from the zero standard. Plot the fluorescence for the standards against the quantity of the standards and draw the best fit curve. To quantify the amount of protein in the samples, find the fluorescence value for the samples on the y-axis and extend a horizontal line to the standard curve. At the intersection point extend a vertical line to the x-axis and read the corresponding standard value. Note: If the samples have been diluted, the value read from the standard curve must be multiplied by the dilution factor.



Example standard curve.
The standard curve at right is provided for demonstration only. You should make a standard curve should be made every time an experiment is performed.

Appendix

Section A: Troubleshooting Guide

I. Buffer Compatibility and Contaminating Substances

A number of common contaminants have been tested with ProStain, and most are well tolerated; however, samples containing high concentrations of free amines are not recommended.

Contaminant	Final Concentration in the assay	Concentration in 100 µl of sample	Result
Sodium chloride	20 mM	20 mM	OK
Sodium chloride	2 mM	4 mM	OK
Tris	10 mM	20 mM	OK
Ammonium sulfate	5 mM	10 mM	OK
Tween	0.001%	0.002%	Not recommended
Triton	0.001%	0.002%	Not recommended
SDS	0.04%	0.08%	OK

* BSA standards were assayed in the presence or absence of contaminants at the indicated final concentrations. Equivalent concentrations in 100 µl sample volumes are also listed. This is not a complete list of contaminants. To determine your buffer compatibility, prepare two BSA curves – one in the same buffer as your samples and one with the supplied Dilution Buffer to determine if there is any interference.

II. Protein-to-Protein Variation

ProStain determines the protein concentration of a sample relative to a BSA standard curve. If you are quantitating recombinant or purified protein rather than a protein extract, accuracy may be improved by using the same protein at a known concentration to make the standard curve, if available.

III. Excitation and Emission Filters

The excitation and emission maxima for Dye Reagent bound to protein are 503 and 602 nm, respectively. We recommend using filters with the following ranges: 485-525 nm Excitation and 575-650 nm Emission.

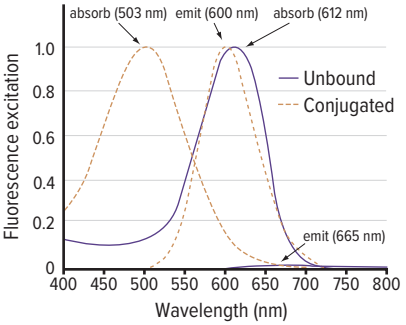


Figure 1: Absorption/emission spectra of free/bound dye.

Normalized absorption and emission spectra of free (solid lines) and conjugated dye (dotted lines) in phosphate buffer of pH 7.2.

Section 4: Technical Services

If you need assistance at any time, please call or send an email to Active Motif Technical Service at one of the locations listed below.

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