

# ProStain™ Protein Quantification Kit

(version C1)

Catalog No. 15001

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## Introduction

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Active Motif's ProStain™ Protein Quantification Kit simplifies protein quantification by providing highly sensitive detection reagents in a convenient and 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 shifts, fast reaction times 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 hrs. 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.

## Kit Components and Storage

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

## Preparation of Reagents

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### 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 rxn
Dye Reagent Stock Soln	110 µl	550 µl	1.1 ml
Distilled H <sub>2</sub> O	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<sub>2</sub>O in the provided tube to make a 1 mg/ml solution. This stock solution can be stored at -20°C for 6 months.

# Protein Quantification Kit Protocol

**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.)
 

**Note:** For higher sensitivity, set up one or both BSA Standard Curve(s) using a 10-fold dilution of the BSA solution (final concentrations of 1.0, 0.5, 0.25, 0.125, 0.063, 0.032, 0.015 and 0.0 µg/ml).
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	9	10	11	12
<b>A</b>	10.0 (1.0) µg/ml	10.0 (1.0) µg/ml	-	-	-	-	-	-	-	-	-	-
<b>B</b>	5.0 (0.5) µg/ml	5.0 (0.5) µg/ml	-	-	-	-	-	-	-	-	-	-
<b>C</b>	2.5 (0.25) µg/ml	2.5 (0.25) µg/ml	-	-	-	-	-	-	-	-	-	-
<b>D</b>	1.25 (0.125) µg/ml	1.25 (0.125) µg/ml	-	-	-	-	-	-	-	-	-	-
<b>E</b>	0.63 (0.063) µg/ml	0.63 (0.063) µg/ml	-	-	-	-	-	-	-	-	-	-
<b>F</b>	0.32 (0.032) µg/ml	0.32 (0.032) µg/ml	-	-	-	-	-	-	-	-	-	-
<b>G</b>	0.15 (0.015) µg/ml	0.15 (0.015) µg/ml	-	-	-	-	-	-	-	-	-	-
<b>H</b>	Blank	Blank	-	-	-	-	-	-	-	-	-	-

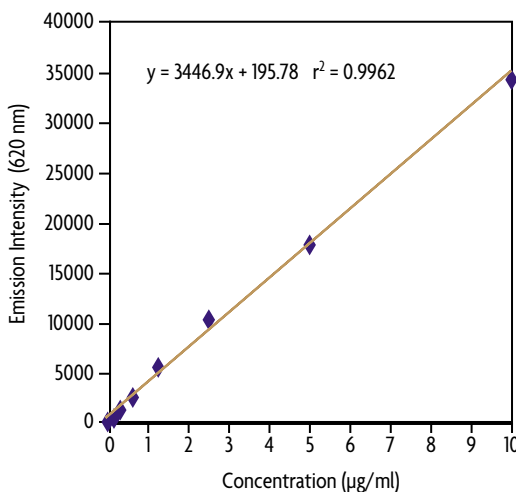
12. **Sample wells:** For protein determination of unknown samples, prepare a series of dilutions with the Dilution Buffer, for example: 1:50, 1:100 and 1:200. Pipette 100  $\mu\text{l}$  into each well. Duplicates of each sample are recommended.
13. Add 100  $\mu\text{l}$ /well of **Dye Reagent Working 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 before then.
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  $\mu\text{s}$ .)
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 curve:

The following standard curve is provided for demonstration only. A standard curve should be made every time an experiment is performed.



### References

1. Hoefelschweiger B.K., Duerkop A. and Wolfbeis O.S. (2005) *Anal. Biochem.* 344: 122-129.

## Appendix

### Section A. Troubleshooting

#### 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 $\mu$ l of sample	Result
Sodium Chloride	20 mM	40 mM	OK
Magnesium 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  $\mu$ l sample volumes are also listed. This is not a complete list of contaminants. To determine 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 a 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.

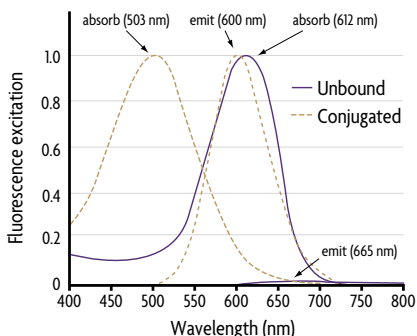


Figure 1: Absorption/emission spectra of free/bound dye. Normalized absorption and emission spectra of free (solid lines) and conjugated dye (dotted lines) in phosphate buffer of pH 7.2.

## Section B. Related Products

TransAM™ Family Kits	Units	Catalog No.
TransAM™ AP-1 Family	2 x 96-well plates	44296
TransAM™ GATA Family	2 x 96-well plates	48296
TransAM™ HNF Family	2 x 96-well plates	46296
TransAM™ IRF Family	2 x 96-well plates	45296
TransAM™ MAPK Family	2 x 96-well plates	47296
TransAM™ Flexi NFκB Family	2 x 96-well plates	43298
TransAM™ NFκB Family	2 x 96-well plates	43296
TransAM™ STAT Family	2 x 96-well plates	42296

### Sandwich ELISAs

FunctionELISA™ IκBα	1 x 96-well plates	48005
	5 x 96-well plates	48505
FunctionELISA™ TRAIL	1 x 96-well plates	48010
	5 x 96-well plates	48510
FunctionELISA™ Cytochrome c	1 x 96-well plates	48006
	5 x 96-well plates	48506
NR Sandwich AR	1 x 96-well plates	49196
	5 x 96-well plates	49696
NR Sandwich ER	1 x 96-well plates	49296
	5 x 96-well plates	49796
NR Sandwich PR	1 x 96-well plates	49396
	5 x 96-well plates	49896

### Chromatin Immunoprecipitation

ChIP-IT™ Kit	25 reactions	53001
ChIP-IT™ w/o controls	25 reactions	53004
ChIP-IT™ Shearing Kit	10 reactions	53002
ChIP-IT Enzymatic	25 reactions	53006
ChIP-IT™ Enzymatic w/o controls	25 reactions	53007
Enzymatic Shearing Kit	10 reactions	53005
Salmon Sperm DNA/ Protein G agarose	25 reactions	53003

### Co-Immunoprecipitation

Nuclear Complex Co-IP Kit	50 reactions	54001
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### Sample Preparation

Nuclear Extract Kit	100 reactions	40010
	400 reactions	40410
Mitochondrial Fractionation Kit	100 reactions	40015
GAPDH Whole-cell Normalization Kit	1 x 96-well plate	48007
	5 x 96 well-plates	48507

### Fluorescent Detection

CE Dye 503	1 kit	15101
CE Dye 540	1 kit	15102

## Technical Services

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If you need assistance at any time, please call Active Motif Technical Service at one of the numbers listed below.

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