Kakkoi Protein Blue™

Bradford Protein Assay Reagent - 5X Concentrate

USER MANUAL

Product	Kakkoi Protein BlueTM Bradford Protein Assay Reagent
Format	5X concentrate
Net volume	400 mL
Storage	2-8 °C; protect from light
Application	Quantitative determination of total protein in aqueous laboratory samples



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For laboratory and professional use. Use in diagnostic workflows requires appropriate local validation, quality systems, and regulatory authorization.

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1. Product overview

Kakkoi Protein BlueTM is a Coomassie Brilliant Blue G-250 dye-binding reagent for rapid colorimetric measurement of protein concentration. The product is supplied as a 5X concentrate. For routine Bradford assays, one volume of concentrate is diluted with four volumes of purified water to prepare the 1X working reagent.

The assay is suitable for research, academic, biotechnology, pharmaceutical, food, environmental, industrial and professional laboratory workflows. Clinical or hospital laboratories may use the reagent for appropriately validated laboratory applications; diagnostic claims should not be made unless the specific procedure has been validated and authorized under applicable regulations.

2. Assay principle

Under acidic conditions, Coomassie Brilliant Blue G-250 changes its spectral properties after binding to proteins. Protein-dye complex formation produces an increase in absorbance near 595 nm. The magnitude of the signal is compared with a protein standard curve prepared under the same assay conditions. Because different proteins can produce different color yields, the most appropriate standard is a purified protein that resembles the sample protein composition whenever practical.

3. Reagent preparation and storage

3.1 Preparation of 1X working reagent

Mix 1 part Kakkoi Protein BlueTM 5X concentrate with 4 parts distilled or deionized water.

- Example: combine 20 mL of 5X concentrate with 80 mL of purified water to obtain 100 mL of 1X reagent.
- Mix gently until uniform. Avoid excessive foaming.
- If visible particulates are present, clarify the working reagent using an appropriate low-protein-binding filter or equivalent laboratory filtration step.
- Prepare only the volume required for routine use whenever possible.

3.2 Storage

- Store the 5X concentrate at 2-8 °C and protect it from direct light.
- Keep the container tightly closed when not in use.
- Do not use reagent showing unexpected precipitation, contamination, leakage, or marked deterioration in assay performance.
- Allow refrigerated reagent to equilibrate toward room temperature before mixing and dispensing, unless an internally validated SOP specifies otherwise.

4. Materials required

- Spectrophotometer or microplate reader capable of measuring absorbance at approximately 595 nm.
- Matched cuvettes or compatible clear microtiter plates.
- Adjustable pipettes and clean disposable tips suitable for the stated assay volumes.
- Protein standard such as bovine serum albumin (BSA), bovine gamma globulin (IgG), or a more representative purified protein.
- Distilled or deionized water.
- Clean test tubes, microcentrifuge tubes, or microplate wells as appropriate.
- Optional microplate mixer or gentle plate-shaking capability.

5. Standard cuvette procedure using 1X reagent

1. Prepare a blank and a series of at least 3-5 protein standards covering the expected concentration range of the samples.
2. Pipette 100 µL of each standard, blank and unknown sample into separate clean tubes. Duplicate or triplicate measurements are recommended.
3. Add 5.0 mL of 1X Kakkoi Protein BlueTM working reagent to each tube.
4. Mix immediately and consistently.
5. Incubate at room temperature for at least 5 minutes. Keep incubation time consistent across standards and samples; complete readings within 1 hour.
6. Measure absorbance at 595 nm against the reagent blank.
7. Construct a standard curve and calculate unknown protein concentrations, correcting for any sample dilution.

6. Microassay procedure using 5X concentrate

This higher-sensitivity format uses a larger sample-to-reagent ratio and the 5X concentrate directly. It is useful when protein concentrations are below the convenient range of the standard cuvette procedure.
1. Prepare a blank and an appropriate series of low-concentration protein standards.
2. Pipette 800 µL of each standard or sample into separate clean tubes.
3. Add 200 µL of Kakkoi Protein BlueTM 5X concentrate.
4. Mix immediately and thoroughly.
5. Incubate at room temperature for at least 5 minutes and no longer than 1 hour before measurement.
6. Read absorbance at 595 nm.
7. Determine sample concentration from a standard curve prepared using the same volume ratio and matrix.

7. Microtiter plate procedures

Microplate performance can differ from cuvette performance because optical path length, well geometry and sample-to-reagent ratios are different. Always generate a fresh standard curve in the same plate format and under the same conditions used for the unknown samples.

7.1 Standard Procedure for Microtiter Plates using 1X reagent

1. Prepare the 1X working reagent by mixing 1 part 5X concentrate with 4 parts distilled or deionized water.
2. Prepare a blank and 3-5 or more protein standards spanning the intended working range. A practical starting range for BSA is approximately 0.05-0.5 mg/mL, but the usable range should be verified on the specific reader and plate type.
3. Dispense 10 µL of each standard and sample into separate microtiter plate wells. Run replicates whenever possible.
4. Add 200 µL of 1X working reagent to each well.
5. Mix thoroughly using a microplate mixer, gentle plate shaking, or careful pipette mixing without introducing bubbles.
6. Incubate at room temperature for at least 5 minutes. Maintain comparable timing among wells and measure within 1 hour.
7. Read absorbance at 595 nm.

8. Subtract the blank response and calculate unknown concentrations from the standard curve.

7.2 Microassay Procedure for Microtiter Plates using 5X concentrate

1. Prepare a blank and a suitable series of low-concentration protein standards.
2. Dispense 160 µL of each standard or sample into separate wells.
3. Add 40 µL of Kakkoi Protein BlueTM 5X concentrate to each well.
4. Mix immediately and consistently while minimizing bubbles.
5. Incubate at room temperature for at least 5 minutes and measure within 1 hour.
6. Read absorbance at 595 nm.
7. Use a standard curve prepared with the same assay ratio to calculate protein concentration.

8. Data analysis and standard curves

- Prepare the blank and standards in the same solvent or buffer matrix as the samples whenever feasible.
- Subtract the mean blank absorbance from all standards and unknowns.
- Plot corrected absorbance versus protein concentration.
- Use only the concentration range that provides an acceptable calibration relationship for the chosen protein standard and instrument.
- Dilute samples that exceed the validated calibration range and repeat the measurement.
- Report the standard protein used, dilution factor, assay format, wavelength and number of replicates.

9. Interferences and sample compatibility

Bradford assays can be affected by substances that alter dye binding, protein structure, solution acidity or optical absorbance. Detergents are a common source of interference, although tolerance depends strongly on the detergent type, concentration, sample-to-reagent ratio and protein composition.

Potential interferent	Possible effect	Recommended action
Detergents (e.g., SDS, Triton-type, Tween-type)	May increase background or alter dye-protein binding.	Match matrix in standards, dilute sample, or validate a detergent-compatible alternative.
Strongly basic buffers / high pH	Can reduce the acidic conditions needed for proper color development.	Dilute or adjust sample conditions while preserving protein solubility.
High concentrations of salts or chelators	May alter protein-dye interactions in some matrices.	Run matrix-matched controls and recovery checks.
Colored or turbid samples	May contribute non-specific absorbance at 595 nm.	Use appropriate sample blanks, clarify samples where scientifically justified, or use an alternative method.
Reducing agents and other additives	Compatibility varies with concentration and protein.	Validate by comparing matrix-matched and water-based standard curves.

10. Troubleshooting

Observation	Likely cause	Suggested action
Low absorbance for standards and samples	Incorrect reagent dilution, degraded reagent, wrong wavelength, insufficient mixing.	Check 1X preparation, reagent condition, instrument wavelength and pipetting sequence.
High blank	Contaminated waterware/reagent, interfering matrix, bubbles or optical artifacts.	Prepare fresh blank, inspect wells/cuvettes, remove bubbles and use clean consumables.
Poor replicate precision	Inconsistent timing, incomplete mixing or pipetting error.	Use calibrated pipettes, consistent mixing and a fixed timing sequence.
Nonlinear standard curve	Standards outside useful range or inappropriate curve model.	Prepare fresh standards and restrict calculations to the validated working range.
Unexpected sample result	Matrix interference or protein-dependent dye response.	Use matrix-matched standards, spike-recovery checks, dilution tests or an alternative protein assay.

Detailed Interfering reagents and chemicals

https://www.researchgate.net/publication/227021616_Protein_Content_Quantification_by_Bradford_Method

11. Safety and handling

Kakkoi Protein BlueTM is an acidic dye reagent intended for trained laboratory personnel. Wear suitable laboratory coat, protective gloves and eye protection. Avoid contact with skin and eyes, and do not ingest. Handle spills and waste according to the current product Safety Data Sheet (SDS), institutional procedures and applicable local regulations.

Important: The SDS supplied for Kakkoi Protein BlueTM is the controlling document for hazard classification, first aid, storage, transport and disposal. Do not rely on legacy safety information from another manufacturer's reagent because solvent composition and hazard classification may differ.

12. References

1. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248-254.
2. Compton SJ, Jones CG. Mechanism of dye response and interference in the Bradford protein assay. Anal Biochem. 1985;151:369-374.
3. Kruger NJ. The Bradford method for protein quantitation. In: Walker JM, ed. The Protein Protocols Handbook. 3rd ed. Humana Press; 2009.