

ClearBand Bradford Reagent Troubleshooting Guide

500 ml

Cat No: BR05

Use this guide for common protein-quantification problems with ClearBand Bradford Reagent. The assay uses Coomassie Brilliant Blue G-250, is read at 595 nm, and requires a fresh standard curve for each assay because the color response is non-linear with increasing protein concentration.

Before You Start - Quick Checks

1. Store at 4°C; warm only the required amount to room temperature before use.
2. Mix the reagent by gentle inversion - do not shake.
3. Prepare a BSA standard curve for every assay and include a blank.
4. Keep the test-tube and microplate formats separate because their sample and reagent volumes are different.

Problem	Most likely cause	Recommended action
Standard curve is poor or non-linear	Bradford color response is inherently non-linear, or standards may have been prepared incorrectly.	Prepare the BSA standards exactly as specified and fit unknowns only against the standard curve generated in the same assay.
Unknown protein concentration is outside the useful range	The sample may be below or above the stated 25-1500 µg/mL assay range.	Adjust the sample concentration and repeat so the reading can be interpreted within the standard-curve range.
Replicates show high variability	Pipetting, mixing, or incubation may differ between replicates.	Run standards and unknowns at least in triplicate and use identical volumes, mixing, and 10-minute room-temperature incubation.
Reagent foams during setup	The bottle may have been shaken instead of gently inverted.	Mix the bottle by gentle inversion only, remove the required amount, and avoid vigorous agitation before dispensing.
Absorbance values are unexpectedly low	The wrong wavelength, insufficient reagent, or wrong sample volume may have been used.	Read at 595 nm and verify the format-specific volumes before repeating the assay.
Blank-corrected values are inconsistent	Blank subtraction may have been omitted or a different blank may have been used.	Average the blank replicates and subtract the blank 595-nm value from every standard and unknown reading.

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Problem	Most likely cause	Recommended action
Test-tube results do not match microplate results	Volumes from the two protocols may have been mixed.	For test tubes use 20 µL sample plus 1 mL reagent; for microplate use 30 µL sample plus 250 µL reagent and 30 seconds of plate shaking.
BSA standards give unexpected concentrations	The stock or serial standard preparation may be incorrect.	Prepare the 2 mg/mL BSA stock from 4 mg BSA in 2 mL ultrapure water and rebuild the standard series from the datasheet table.
Signal changes between assays	A previous standard curve may have been reused instead of creating one with the current assay.	Generate a new BSA standard curve each time, as required by the non-linear Bradford response.
Reagent was used directly from 4°C	The protocol calls for warming the required amount to room temperature before use.	Remove only the amount needed, allow it to reach room temperature, then perform the assay.
Incubation-dependent drift is observed	Samples may not have been incubated for the same 10-minute period.	Time all standards and unknowns consistently and read after the same room-temperature incubation.
Plate readings vary across wells	Mixing after reagent addition may be incomplete.	For the microplate format, mix thoroughly on a plate shaker for 30 seconds before the 10-minute incubation.

Still having trouble? Recheck the BSA standards, assay format, 595-nm reading, blank subtraction, triplicates, and the exact sample-to-reagent volumes before repeating unknown measurements.