Protein Detection and Analysis

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DOMINIQUE DUTSCHER SAS

Biotium

TrueBlack® WB Blocking Buffer Kit

A superior blocking solution for fluorescent western blots

Non-specific signal in WB can arise from multiple sources, including antibody cross-reactivity with off-target proteins, non-specific antibody adsorption to the membrane, and membrane autofluorescence. Often, labeling antibodies with fluorescent dyes can decrease antibody specificity which leads to increased background fluorescence. The TrueBlack® WB Blocking Buffer Kit blocks background from multiple sources including charged dye conjugates (Fig. 1). TrueBlack® blocking buffer is especially advantageous for phosphoprotein detection, significantly improving specificity compared to conventional blocking buffers (Fig. 2).

Key Features

- Blocks non-specific protein binding, reducing background fluorescence over the entire membrane
- Reduces antibody cross-reactivity, eliminating non-specific bands
- Works as well or better than LI-COR's Odyssey® or Intercept® Blocking Buffers, at a lower cost per membrane
- Suitable for phosphoprotein detection

Compatibility

- Use with PVDF or nitrocellulose membranes
- For visible or near-IR fluorescent western
- Contains no mammalian protein, can be used with anti-goat and anti-bovine antibodies
- **NOT for use with HRP-based chemiluminescent detection**

TrueBlack® blocks non-specific background from negatively charged fluorescent dyes

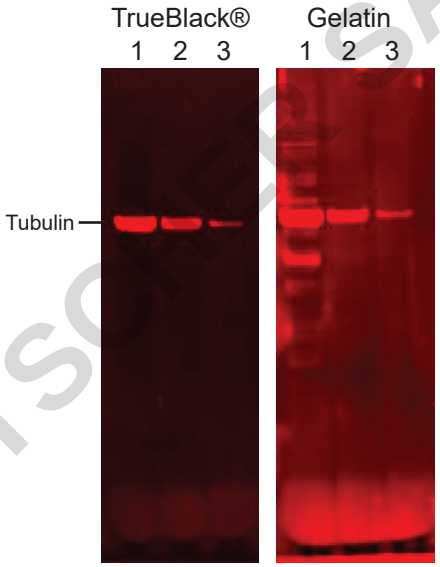


Figure 1. Western blot detection of tubulin with primary mouse anti-tubulin and secondary Alexa Fluor® 790 goat anti-mouse antibodies. Membranes were blocked with fish gelatin or TrueBlack® WB Blocking Buffer. The Alexa Fluor® 790 conjugate showed non-specific binding to PVDF and proteins, which was blocked by TrueBlack® WB Blocking Buffer. Lanes 1-3: 10 ug, 1 ug, or 0.1 ug HeLa cell total protein.

Unrivaled signal specificity for phosphoproteins compared to Odyssey® or gelatin

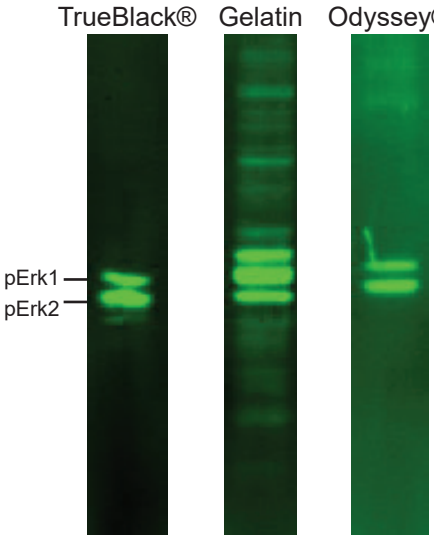


Figure 2. Western blot detection of phospho-Erk1/2 in PDGF-stimulated NIH-3T3 cell lysate. Membranes were blocked with fish gelatin, LI-COR® Odyssey® TBS Blocking Buffer, or TrueBlack® WB Blocking Buffer. Rabbit anti-pErk1/2 and CF®680R donkey anti-rabbit antibodies were used for detection. TrueBlack® WB Blocking Buffer gave lower background fluorescence and better specificity.

TrueBlack® WB Blocking Buffer Kit

Cat. #	Product	Size
23013-T	TrueBlack® WB Blocking Buffer Kit	For 10 membranes
23013		For 50 membranes

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TrueBlack is a registered trademark of Biotium; Alexa Fluor is a registered trademark of Thermo Fisher Scientific; LI-COR and Odyssey are registered trademarks of LI-COR Biosciences.

VersaBlot™ Total Protein Normalization Kits

A highly linear and reversible protein prestain for in-gel detection or western normalization

VersaBlot™ Total Protein Normalization Kit allows simple and reversible labeling of purified proteins or cell lysates with near-infrared CF® dyes. The stain is linear over a wide dynamic range with sensitive detection down to ~1 ng/band. To label a protein sample, simply mix the dye and buffer into your protein solution, wait 30 minutes, and then separate the labeled protein sample by SDS-PAGE. After electrophoresis, the bands can be visualized using a fluorescent gel scanner, eliminating the need for gel staining. Labeled proteins on SDS-PAGE gels may be transferred to membranes for western blot normalization. If desired, reversal solution can be used to remove membrane staining for downstream multicolor WB analysis.

Key Features

- Superior linearity over a wide dynamic range for western blot normalization compared to housekeeping proteins
- Reversible staining for downstream multicolor western blotting
- Highly sensitive protein quantitation down to ~1 ng/band on PAGE gels or western membranes
- Simple protocol for total protein labeling of purified samples or cell lysates
- Choice of 2 bright near-IR dyes: CF®680T and CF®770T
- Compatible with Typhoon™, Odyssey®, and other fluorescence imagers

Superior linearity over a wide dynamic range relative to housekeeping proteins

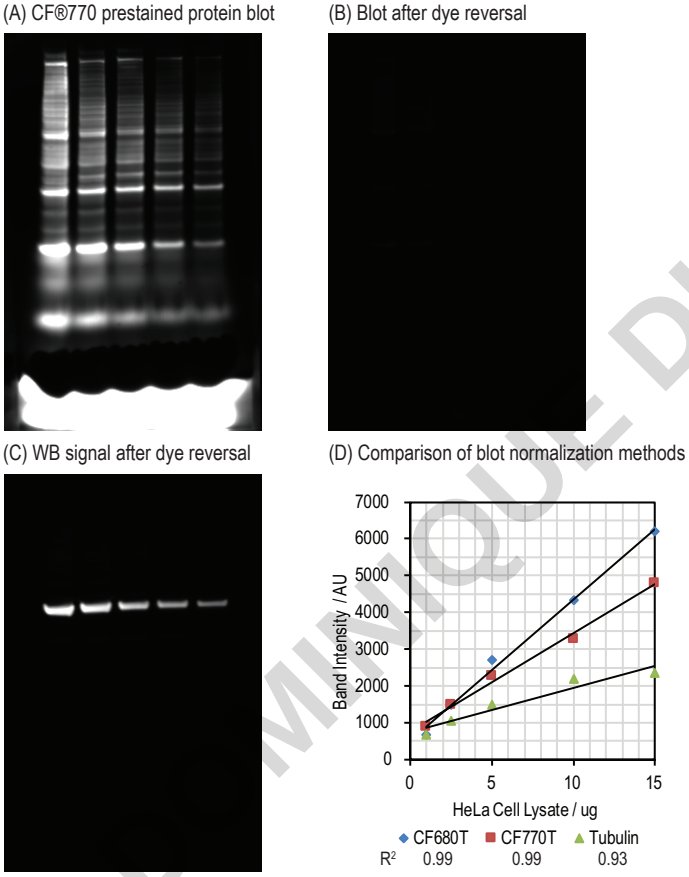


Figure 1. VersaBlot™ Total Protein Normalization Kits for WB normalization. (A) Serial dilutions of HeLa cell lysate were labeled with VersaBlot™ CF®770T kit and transferred to PVDF membrane. (B) Fluorescence following reversal protocol. (C) Tubulin detection via CF®770 conjugated secondary antibody after reversal. (D) Plots of band intensity vs. protein content for CF®680T and CF®770T labeled lysate compared to tubulin WB. The VersaBlot™ Total Protein Normalization Kits showed better linearity compared to the antibody-based detection of housekeeping proteins.

Unrivaled precision for in-gel protein quantitation compared to REVERT™ Total Protein Stain

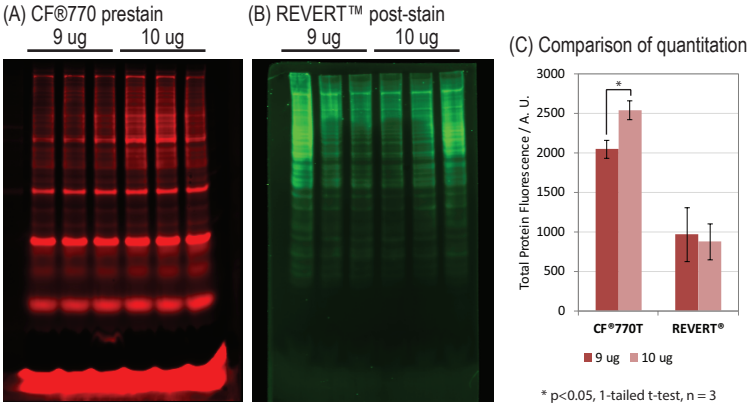


Figure 2. HeLa cell lysate was labeled with the VersaBlot™ CF®770T kit or left unlabeled. Lysates were loaded on SDS-PAGE gels at 9 ug or 10 ug total protein per lane and transferred to PVDF membranes. Proteins were stained with (A) VersaBlot™ CF®770T signal or (B) REVERT™ Total Protein Stain and imaged on an Odyssey® Infrared Imaging System. (C) CF®770T prestain demonstrated significant difference in signal while the REVERT™ kit was not able to quantitate a 10% difference in protein content.

VersaBlot™ Total Protein Normalization Kits

Cat. #	Product	Size
33025-T	VersaBlot™ CF®680T Total Protein Normalization Kit	100 labelings
33025		500 labelings
33026-T	VersaBlot™ CF®770T Total Protein Normalization Kit	100 labelings
33026		500 labelings

VersaBlot is a trademark of Biotium; REVERT is a trademark of LI-COR Biosciences; Odyssey and LI-COR are registered trademarks of LI-COR Biosciences; Typhoon is a trademark of GE Healthcare.

EXEMPLAIRE UNIQUE DUTSCHER SAS

Near-IR CF® Dye Conjugates for Western Blotting

Near-IR CF® Dyes offer highly sensitive detection over a wide linear range for multiplex western blotting

Near-infrared (near-IR) western blot detection is highly sensitive, and offers advantages of wider linear range and multiplexing capability compared to chemiluminescence detection. Biotium's near-IR CF® dyes offer low non-specific binding and are less prone to aggregation induced quenching than other near-IR dyes, resulting in more soluble and brighter conjugates. We offer a wide selection of primary and highly cross-adsorbed secondary antibodies conjugated to our exceptional near-IR CF® dyes for western blot. We also offer a wide selection of HRP secondary antibody conjugates for chemiluminescence detection.

Key Features of CF® dyes

- Exceptionally bright and stable
- Primary and secondary antibody conjugates available
- Superior signal-to-noise for bioconjugates
- Compatible with LI-COR® Odyssey® and other near-IR imagers

Highly Cross Adsorbed Secondary Antibodies Conjugated to HRP and Near-IR CF® Dyes for Western Blotting

Conjugate (Ex/Em)	HRP	CF®680 (681/698 nm)	CF®680R (680/701 nm)	CF®750 (755/777nm)	CF®770 (770/797 nm)	CF®790 (784/806 nm)	CF®800 (797/816 nm)
Donkey Anti-Goat	---	20060	20196	20362	20277	20345	20834
Donkey Anti-Guinea Pig	---	20241	---	---	20242	---	---
Donkey Anti-Mouse	20404	---	20194	---	---	20363	20835
Donkey Anti-Rabbit	20405	20418	20195	20298	20484	20344	20833
Donkey Anti-Rat	---	20417	---	20857	---	---	---
Donkey Anti-Sheep	---	20062	---	---	---	---	---
Donkey Anti-Human	---	20278	---	---	200279	---	---
Goat Anti-Chicken	20474	---	---	---	---	---	---
Goat Anti-Guinea Pig	---	20499	20498	---	20500	---	---
Goat Anti-Mouse	20401	20065	20192	20463	20077	20342	20831
Goat Anti-Rabbit	20403	20067	20193	---	20078	20343	20832
Goat Anti-Rat	20406	20069	---	---	20383	---	---
Goat Anti-Human	20470	20287	---	---	20288	---	---
Rabbit Anti-Mouse	---	20061	---	---	---	---	---

Don't see what you're looking for? Contact us at btinfo@biotium.com! We may be able to perform a custom labeling for you.

Peacock™ Prestained Protein Markers

Peacock™ Prestained Protein Markers are three-color protein ladders that allow visual monitoring of protein separation during SDS-PAGE and protein transfer to membranes for western blotting. The ladders are ready-to-use with no heating or other preparation required. Peacock™ Prestained Protein Marker contains 10 visible bands ranging from 10 kDa to 180 kDa. Meanwhile, our Peacock™ Plus Prestained Protein Marker contains 12 visible bands over a broader range of 8 kDa to 245 kDa.

Peacock™ Prestained Protein Markers

Cat. #	Product	Size
21530-50uL	Peacock™ Prestained Protein Marker	50 uL
21530-500uL		500 uL
21531-50uL	Peacock™ Plus Prestained Protein Marker	50 uL
21531-500uL		500 uL

Other Western Blotting Reagents

Biotium offers a wide selection of traditional western blotting reagents including blocking reagents, buffers, reductants, and protein stains.
Visit www.biotium.com to view our full catalog.

Other Western Blotting Reagents

Cat. #	Product	Size
22010	10X Fish Gelatin Blocking Agent	100 mL
22011	Fish Gelatin Powder	2 X 50 g
22014	Bovine Serum Albumin, 30% Solution	100 mL
22013	Bovine Serum Albumin Fraction V	50 g
22012	Dry Milk Powder	4 X 25 g
22020	10X Phosphate Buffered Saline (PBS)	4 L Cubitainer®
22002	Tween®-20	50 mL
22001	Ponceau S Solution	1 L
91050	DTT	1 g
91049	TCEP	1 g

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DOMINIQUE DUTSCHER SAS

Rapid and ready-to-use protein stains for colorimetric or fluorescent detection

One-Step stains can be applied to PAGE gels to stain proteins in a single-step without fixation or washing. One-Step stains offer safer handling and disposal compared to Coomassie and other stains because they are entirely aqueous-based and do not contain hazardous methanol or acetic acid.

Biotium offers three versions of One-Step stains for different visualization methods. With One-Step Blue®, proteins can be detected by visible blue staining, or by near-infrared fluorescence. One-Step Lumitein™ Protein Gel Stain is a red fluorescent protein gel stain that is able to be detected using a UV light box, laser gel scanner, or blue light illuminator like Gel-Bright™. One-Step Lumitein™ UV is optimized for maximum sensitivity on a UV transilluminator.

One-Step Blue®

- Faster and simpler than tedious Coomassie staining
- Colorimetric blue or near-IR fluorescence detection
- Detection limit of ~10-20 ng of protein.

One-Step Lumitein™

- Easier to use and lower cost than SYPRO® Ruby gel stain for fluorescence imaging
- Detect with a fluorescence-based gel imager (such as Typhoon®) or a UV gel box
- Detect as little as 1-10 ng of protein

One-Step Lumitein™ UV

- Same convenience and sensitivity as One-Step Lumitein™ optimized for UV-based detection
- Does not shrink gel, unlike Oriole® fluorescent gel stain (Fig. 3)

One-Step Blue®

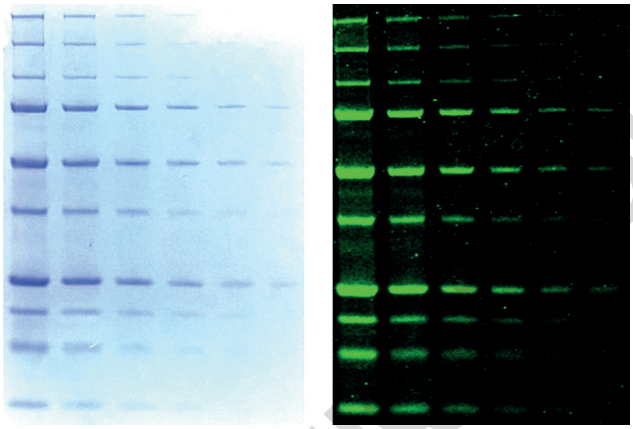


Figure 1. A protein ladder was loaded onto a SDS-PAGE gel in 2-fold dilutions and stained with One-Step Blue®. One-Step Blue® staining can be visualized as visible blue bands (left), or near-IR fluorescence (right).

One-Step Lumitein™

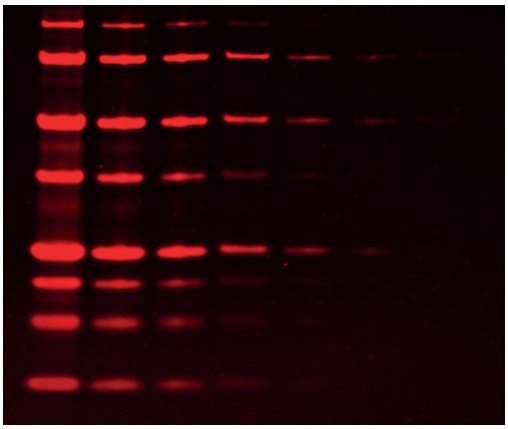


Figure 2. A protein ladder was loaded onto an SDS-PAGE gel in 2-fold dilutions, and the gel was stained with One-Step Lumitein™. One-Step Lumitein™ fluorescent staining can be visualized on a fluorescence-based gel imager or a UV gel box.

One-Step Lumitein™ UV vs Oriole®

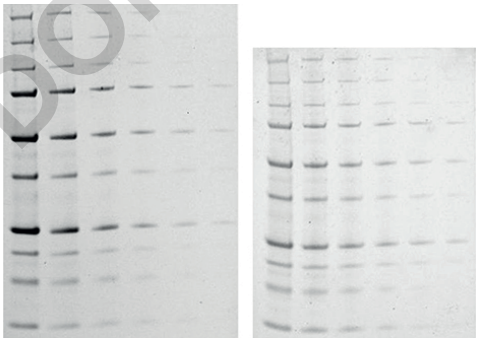


Figure 3. Identical SDS-PAGE gels were stained with One-Step Lumitein™ UV (left), or Oriole® (right). The gels began as the same size, but staining with Oriole® caused gel shrinkage

One-Step Protein Gel Stains

Cat. #	Product	Size
21003-1L	One-Step Blue® Protein Gel Stain	1 L
21004-1L	One-Step Lumitein™ Protein Gel Stain	1 L
21004-4L		4 L
21005-1L	One-Step Lumitein™ UV Protein Gel Stain	1 L
21005-4L		4 L

Lumitein is a trademark of Biotium; One-Step Blue is a registered trademark of Biotium; Oriole is a registered trademark of Bio-Rad Laboratories; Odyssey is a registered trademark of LI-COR Biosciences; Typhoon is a registered trademark of GE Healthcare.

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GloMelt™ Kits for Thermal Shift Assay

A rapid and highly sensitive assay that measures protein thermal stability

GloMelt™ dye undergoes fluorescence enhancement upon binding to hydrophobic regions of denatured proteins. Therefore the dye can be used to monitor the temperature dependent unfolding of a protein or to measure a protein's thermal stability. These types of assays are known as thermal shift assay, Protein Thermal Shift™, differential scanning fluorimetry, or Thermofluor assay.

The thermal shift assay is a rapid and inexpensive technique that quantifies change in protein denaturation temperature. This can be used to screen conditions that affect protein thermal stability, such as protein mutations, ligand binding, and buffer formulations (like pH, salts, detergents, and other additives). The assay is rapid, and can be performed on a quantitative PCR system. The thermal shift method is compatible with high-throughput screening and requires much less protein than methods such as circular dichroism and differential scanning calorimetry.

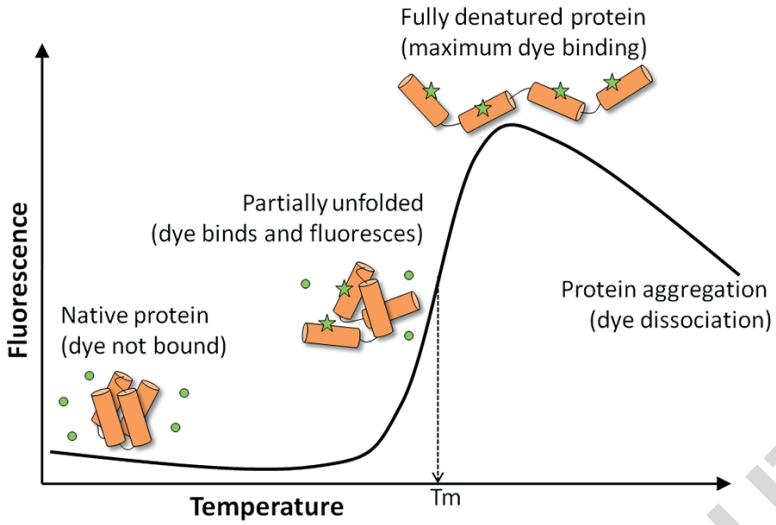


Figure 1. Environmentally-sensitive fluorescent dyes can be used to monitor the temperature dependent unfolding of a protein. The protein's melting temperature (Tm) is a reporter of the protein's thermal stability.

GloMelt™ performs better in the presence of detergent compared to SYPRO® Orange

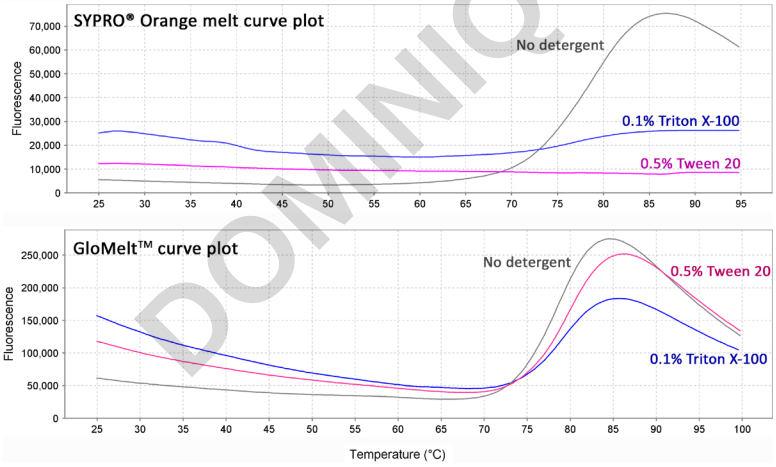


Figure 2. IgG melt curve plots in the presence of detergent. A thermal shift assay was performed on 20 ug IgG in the presence of 5X SYPRO® Orange or 1X GloMelt™ dye, using a QuantStudio™ 5 qPCR system. The presence of detergent inhibited the SYPRO® Orange assay, but did not prevent melt curve detection in the GloMelt™ assay.

Applications

- Optimize buffer formulation for protein stability and storage
- Determine how mutations affect protein stability
- Rapidly screen small molecule drug candidates and other ligands for protein binding

Key Features

- Superior sensitivity for detection in qPCR instruments
- Compatible with reducing agents, common buffers/excipients, and a wide pH range
- Tolerant to high detergent concentrations, unlike SYPRO® Orange (Fig. 2)
- Ideal for high-throughput assays, low reaction volumes, and low protein concentrations
- Improved reproducibility when used with ROX reference dye
- GloMelt™ dye is highly soluble and stable in aqueous buffers

GloMelt™ Thermal Shift Protein Stability Kits

Cat. #	Product	Size
33021-T	GloMelt™ Thermal Shift Protein Stability Kit	200 x 20 uL reactions
33021-1		2000 x 20 uL reactions
33022-T	GloMelt™ Thermal Shift Protein Stability Kit (with ROX)	200 x 20 uL reactions
33022-1		2000 x 20 uL reactions

GloMelt is a trademark of Biotium; QuantStudio and Protein Thermal Shift are trademarks of Thermo Fisher Scientific; SYPRO is a registered trademark of Thermo Fisher Scientific.

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Highly sensitive fluorometric protein assay

AccuOrange™ Protein Quantitation Kit is a highly sensitive fluorescence-based assay for quantitating purified protein samples in 96-well format. AccuOrange™ has greater sensitivity than traditional protein quantitation assays such as BCA, Bradford and Lowry; and shows superior linearity and reproducibility compared to the NanoOrange® protein quantitation assay (Fig. 1). The assay shows minimal variability between different proteins, and has stable fluorescence signal for up to 16 hours.

AccuOrange™ is recommended for quantitating purified protein or antibody samples. The AccuOrange™ assay has low tolerance for non-ionic detergents, and is not recommended for use with cell lysates containing Triton X-100, sodium deoxycholate, CHAPs, or other non-ionic detergents. The assay can tolerate up to 0.01% SDS (final concentration in assay).

AccuOrange™ Features

- Detected in green channel of fluorescence microplate readers
- Wider linear detection range compared to NanoOrange®
- 200 uL microplate assay
- Minimal protein to protein variation
- Fluorescence signal stable for up to 16 hours
- Compatible with reducing agents, amino acids, nucleic acids, and imidazole
- For use with purified protein or antibody samples

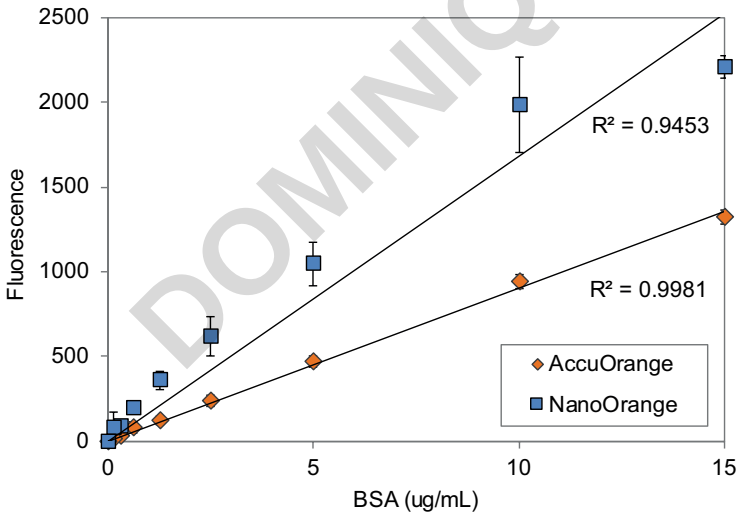


Figure 1. AccuOrange™ shows better linearity and reproducibility compared to NanoOrange® Protein Quantitation assay. BSA titration was performed in triplicate using AccuOrange™ Protein Quantitation Kit or NanoOrange® Protein Quantitation Kit from Thermo Fisher Scientific according to manufacturer's protocol and read on a microplate reader at the recommended wavelengths for each assay. Error bars represent standard deviation of the mean for triplicate samples.

Table 1. Comparison of AccuOrange™ with other protein quantitation assays

Assay Type	Detection Range (Microplate Assay)	Comments
AccuOrange™	**0.1-15 ug/mL**	• **Fluorescence detection (480/598 nm)** • **Highly linear** • **Signal stable for up to 16 hours** • **Compatible with reducing agents** • **Not compatible with detergents**
NanoOrange®	0.1-10 ug/mL	• Fluorescence detection (470/570 nm) • Poor linearity • Fluorescence stable for 6 hours • Compatible with reducing agents • Not compatible with detergents
Modified Lowry	1-1500 ug/mL	• Absorbance detection (750 nm) • Poor linearity • Not compatible with reducing agents • Not compatible with detergents
BCA	20-2000 ug/mL	• Absorbance detection (562 nm) • Highly linear • Signal not stable over time • Not compatible with reducing agents • Compatible with detergents
Bradford (Coomassie)	50-500 ug/mL	• Absorbance detection (595 nm) • Poor linearity • Signal not stable over time • Compatible with reducing agents • Not compatible with detergents
Pierce™ 660 nm	50-2000 ug/mL	• Absorbance detection (660 nm) • Poor linearity • Compatible with reducing agents • Compatible with detergents
A_{280}	50-2000 ug/mL	• Absorbance detection (280 nm) • High protein to protein variability • Contaminants such as nucleic acids can affect results

AccuOrange™ Protein Quantitation Kit

Cat. #	Product	Size
30071-T	AccuOrange™ Protein Quantitation Kit	200 assays
30071		2000 assays

AccuOrange is a trademark of Biotium; Pierce is a trademark of Thermo Fisher Scientific; NanoOrange is a registered trademark of Thermo Fisher Scientific.

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COMMERCIAL DUPLICATION SAS

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