

Thermo Scientific Pierce Protein Assay Selection Guide

Thermo Scientific™ Pierce™ Protein Assay	Pierce 660nm Protein Assay (Product # 22660 and 22662)	BCA Protein Assay (Product # 23225 and 23227)	Micro BCA Protein Assay (Product # 23235)	BCA, Reducing Agent Compatible Assay (Product # 23250 and 23252)	Coomassie Plus Protein Assay (Product # 23236)	Coomassie Protein Assay (Product # 23200)	Modified Lowry Protein Assay (Product # 23240)
Advantages	<ul style="list-style-type: none"> Compatible with reducing agents, chelating agents and detergents Faster and easier to perform than BCA or Coomassie (Bradford) Assays Excellent linearity of color development within the detection range Reaches a stable end point Compatible with Laemmli sample buffer containing bromophenol blue 	<ul style="list-style-type: none"> Faster and easier to perform than the Lowry Protein Assay As accurate as the Lowry Protein Assay and more stable Compatible with most ionic and nonionic detergents (SDS, Triton X-100, etc.) Less protein-to-protein variability than the Coomassie (Bradford) Assay 	<ul style="list-style-type: none"> Measures dilute protein samples in the presence of detergents Easier to perform than the Lowry Protein Assay Less protein-to-protein variability than the Coomassie (Bradford) Assay Excellent linearity of color response within the detection range 	<ul style="list-style-type: none"> Measures protein samples in the presence of reducing agents, chelating agents and detergents Less protein-to-protein variability than the Coomassie (Bradford) Assay Assay protocol utilizes small sample volumes Available in both standard and microplate formats 	<ul style="list-style-type: none"> Lowest protein-to-protein variability of any standard coomassie dye-based (Bradford) Assay Faster than the BCA or Lowry Protein Assays Ready to use: does not require prefiltering or dilution Compatible with reducing agents (BME, DTT) Reaches a stable end-point Superior linear response ranging from 125 to 1,000µg/mL for BSA Best linearity of color response of all coomassie dye-based protein assays 	<ul style="list-style-type: none"> Faster than the BCA or Lowry Protein Assays Ready to use: does not require prefiltering or diluting Compatible with reducing agents (BME, DTT) Reaches a stable end-point Used for quick estimation of protein concentration Used to measure protein in the presence of reducing agents Measures protein of > 3,000Da 	<ul style="list-style-type: none"> Stable, ready-to-use copper solution (no need to make fresh every day) More accurate than the Coomassie (Bradford) Assay Less protein-to-protein variability than the Coomassie (Bradford) Assay Reaches a stable end-point where color absorbance does not drift 100% correlation with the original Lowry Method
Applications	<ul style="list-style-type: none"> Ideal for measuring total protein concentration in samples containing both reducing agents and detergents Used for quick yet accurate estimation of protein Measures peptides as small as 2,500Da 	<ul style="list-style-type: none"> Ideal for measuring detergent-solubilized membrane proteins Measures peptides as small as 2,000Da Can be used to measure protein on solid surfaces such as affinity supports, plastic microplates and membranes 	<ul style="list-style-type: none"> Ideal for measuring dilute protein solutions that do not contain reducing agents Measures peptides as small as 2,000Da 	<ul style="list-style-type: none"> Ideal for measuring total protein concentration in samples containing both reducing agents and detergents Measures peptides as small as 2,000Da 	<ul style="list-style-type: none"> Used for quick estimation of protein concentration Used to measure protein in the presence of reducing agents Measures protein of > 3,000Da 	<ul style="list-style-type: none"> Used for quick estimation of protein concentration Used to measure protein in the presence of reducing agents Measures protein of > 3,000Da 	<ul style="list-style-type: none"> Substitute for "homemade" preparations of the Lowry reagents For the determination of a broad range of proteins after TCA precipitation Compatible with any published method for sample treatment modification of the Lowry Assay
Standard Assay Protocol							
Precautions	<ul style="list-style-type: none"> Use reagent with IDCR (Ionic Detergent Compatibility Reagent) with samples containing ionic detergents like SDS Greater protein-to-protein variability than the BCA Assay 	<ul style="list-style-type: none"> Not compatible with reducing agents such as DTT, β-mercaptoethanol (BME), glucose or ascorbic acid Measure samples within 10 minutes to avoid effects of drifting color 	<ul style="list-style-type: none"> Not compatible with reducing agents such as DTT, β-mercaptoethanol (BME), glucose or ascorbic acid Measure samples within 10 minutes to avoid effects of drifting color 	<ul style="list-style-type: none"> Measure samples within 10 minutes to avoid the effects of drifting color 	<ul style="list-style-type: none"> Interference from ionic and nonionic detergents Greater protein-to-protein variability than the BCA or Lowry Assays 	<ul style="list-style-type: none"> Interference from ionic and nonionic detergents Greater protein-to-protein variability than the BCA and Lowry Assays 	<ul style="list-style-type: none"> Interference from reducing agents, some detergents, copper-chelating agents, carbohydrates and Tris Requires tinted addition of reagents Practical limit of about 20 samples at one time
Reaction Schemes							
Typical Standard Curves Prepared Using Pre-Diluted BSA and BGG Standard Sets							
Detection Range	25-2,000µg/mL	20-2,000µg/mL or 5-250µg/mL	0.5-20µg/mL	125-2,000µg/mL	100-1,500µg/mL or 1-25µg/mL	100-1,500µg/mL or 1-25µg/mL	1-1,500µg/mL
Interfering Substances (Maximum Concentration)	<p>Virtually every protein detection method known exhibits a particular sensitivity to the presence of biochemical reagents in the protein sample. Protein samples typically contain detergents, buffer salts, denaturants, reducing agents, chaotropic agents or antimicrobial preservatives, depending on the preparation technique the protein sample has undergone. These additives can affect the results of an assay. When a component of a protein solution enhances or artificially increases the color response of any assay, the component is considered to be an interfering substance. Substances that do not affect or that marginally affect the results of an assay are known as compatible substances. Interfering substances can affect the assay of protein in one of the following ways:</p> <ol style="list-style-type: none"> They can suppress the response of an assay. They can artificially enhance the response of an assay. They can result in an elevated background measurement. <p>Interference from many common substances can be compensated for in the blank designated for a specific assay. To avoid significant interference, the standard curve must be prepared in the same diluent that is used for the sample. When only a "rough" estimate of protein is needed, a blank-only correction can be used. In this case, a blank is prepared in the diluent of the sample to correct for its raw absorbance. The concentration of the sample is then determined from a standard curve obtained from a series of dilutions of a protein of known concentration prepared in water or saline.</p>	<p>2-D sample buffer¹ undiluted</p> <p>2-Mercaptoethanol 1M</p> <p>ACES, pH 7.8 50mM</p> <p>Acetone 50%</p> <p>Acetonitrile 50%</p> <p>Ammonium sulfate 125mM</p> <p>Aprotinin 2mM</p> <p>Ascorbic acid 500mM</p> <p>Asparagine 40mM</p> <p>Bicline >1M</p> <p>Bis-Tris pH 6.5 50mM</p> <p>Borate (50mM) undiluted</p> <p>pH 8.5</p> <p>B-PER Reagent 1.2</p> <p>B-PER Reagent II 1.2</p> <p>B-PER Reagent PPS 1.2</p> <p>Brij-56 5%</p> <p>Brij-58 5%</p> <p>Bromophenol blue 0.031%</p> <p>CaCl₂ (in 50mM NaOH)</p> <p>Calcium chloride 40mM</p> <p>Cesium bicarbonate 100mM</p> <p>Cetyltrimethylammonium chloride 2.5%</p> <p>CHAPS 5%</p> <p>CHAPS 5%</p> <p>CHAPS 5%</p> <p>CHAPS 5%</p> <p>CHES >50mM</p> <p>Cobalt chloride (in 50mM NaOH)</p> <p>Citrate 350mM</p> <p>Dithioerythritol (DTE) 25mM</p> <p>Dithiothreitol (DTT) 25mM</p> <p>DMF 50%</p> <p>DMSO 50%</p> <p>DTAB 2%</p> <p>EDTA 20mM</p> <p>EGTA 20mM</p> <p>EPPS pH 8.0 100mM</p> <p>Ethanol 20mM</p> <p>Ferric chloride (in 10mM NaOH)</p> <p>Ferric chloride 5mM</p> <p>Glucose 500mM</p> <p>Glutathione 100mM</p> <p>Glycerol (fresh) 10%</p> <p>Glycine-HCl pH 2.8 100mM</p> <p>Guandine-HCl 2.5M</p> <p>HEPES pH 7.5 100mM</p> <p>Hydrides (NaBH₄ or NaCNBH₃)</p> <p>Hydrochloric acid 125mM</p> <p>Imidazole pH 7.0 200mM</p> <p>I-PER Reagent 1.4</p> <p>Laemmli SDS undiluted</p> <p>Laemmli SDS sample buffer¹</p> <p>Leupeptin 80µg/mL</p> <p>Mannitol 100mM</p> <p>Melibiose 500mM</p> <p>MES pH 6.1 125mM</p> <p>Methanol 50%</p> <p>Modified Dulbecco's undiluted</p> <p>MOPS pH 7.2 125mM</p>	<p>2-D sample buffer¹ n/a</p> <p>2-Mercaptoethanol 0.01%</p> <p>ACES, pH 7.8 25mM</p> <p>Acetone 10%</p> <p>Acetonitrile 10%</p> <p>Ammonium sulfate 15mM</p> <p>Aprotinin 10µg/mL</p> <p>Ascorbic acid 0</p> <p>Asparagine 1mM</p> <p>Bicline 20mM</p> <p>Bis-Tris pH 6.5 33mM</p> <p>Borate (50mM) undiluted</p> <p>pH 8.5</p> <p>B-PER Reagent undiluted</p> <p>B-PER Reagent II n/a</p> <p>B-PER Reagent PPS n/a</p> <p>Brij-56 5%</p> <p>Brij-58 1%</p> <p>Bromophenol blue (in 50mM NaOH)</p> <p>Calcium chloride 10mM</p> <p>Cesium bicarbonate 100mM</p> <p>Cetyltrimethylammonium chloride n/a</p> <p>CHAPS 5%</p> <p>CHAPS 5%</p> <p>CHES 100mM</p> <p>Cobalt chloride (in 50mM NaOH)</p> <p>Citrate n/a</p> <p>Cysteine n/a</p> <p>Dithioerythritol (DTE) 1mM</p> <p>Dithiothreitol (DTT) 1mM</p> <p>DMF 1%</p> <p>DMSO 1%</p> <p>DTAB n/a</p> <p>EDTA n/a</p> <p>EGTA n/a</p> <p>EPPS pH 8.0 100mM</p> <p>Ethanol 10%</p> <p>Ferric chloride (in 10mM NaOH)</p> <p>Ferric chloride 10mM</p> <p>Glucose 100mM</p> <p>Glutathione (reduced) n/a</p> <p>Glycerol (fresh) 10%</p> <p>Glycine-HCl pH 2.8 100mM</p> <p>Guandine-HCl 4M</p> <p>HEPES pH 7.5 100mM</p> <p>Hydrides (NaBH₄ or NaCNBH₃) n/a</p> <p>Hydrochloric acid (in 10mM NaOH)</p> <p>Imidazole pH 7.0 50mM</p> <p>I-PER Reagent undiluted</p> <p>Laemmli SDS n/a</p> <p>Laemmli SDS sample buffer¹ n/a</p> <p>Leupeptin 10µg/mL</p> <p>Mannitol n/a</p> <p>Melibiose n/a</p> <p>MES pH 6.1 100mM</p> <p>Methanol n/a</p> <p>Modified Dulbecco's undiluted</p> <p>MOPS pH 7.2 100mM</p> <p>MOPS pH 7.2 100mM</p>	<p>2-D sample buffer¹ n/a</p> <p>2-Mercaptoethanol 1mM</p> <p>ACES, pH 7.8 10mM</p> <p>Acetone 10%</p> <p>Acetonitrile 30%</p> <p>Ammonium sulfate 0</p> <p>Aprotinin 1µg/mL</p> <p>Ascorbic acid n/a</p> <p>Asparagine n/a</p> <p>Bicline 1mM</p> <p>Bis-Tris pH 6.5 16.5mM</p> <p>Borate (50mM) pH 8.5 0.2%</p> <p>B-PER Reagent 1.3</p> <p>B-PER Reagent II 1.4</p> <p>B-PER Reagent PPS 1.4</p> <p>Brij-35 0.63%</p> <p>Brij-56 0.50%</p> <p>Bromophenol blue (in 50mM NaOH)</p> <p>Calcium chloride 1mM</p> <p>CHAPS 5%</p> <p>CHAPS 5%</p> <p>CHES 50mM</p> <p>Cobalt chloride (in 50mM NaOH)</p> <p>CitAB n/a</p> <p>Cysteine 2.5mM</p> <p>Dithioerythritol (DTE) 5mM</p> <p>Dithiothreitol (DTT) 5mM</p> <p>DMF 0.25%</p> <p>DMSO 10%</p> <p>DTAB 10%</p> <p>EDTA 10%</p> <p>EPPS pH 8.0 100mM</p> <p>Ethanol 5mM</p> <p>Ferric chloride (in 10mM NaOH)</p> <p>Ferric chloride 5mM</p> <p>Glucose 100mM</p> <p>Glutathione (reduced) 10mM</p> <p>Glycerol (fresh) 5%</p> <p>Glycine-HCl pH 2.8 50mM</p> <p>Guandine-HCl¹ 1.5M</p> <p>HEPES pH 7.5 200mM</p> <p>Hydrides (NaBH₄ or NaCNBH₃) n/a</p> <p>Hydrochloric acid (in 10mM NaOH)</p> <p>I-PER Reagent n/a</p> <p>Laemmli SDS n/a</p> <p>Laemmli SDS sample buffer¹ n/a</p> <p>Leupeptin n/a</p> <p>Mannitol n/a</p> <p>Melibiose n/a</p> <p>MES pH 6.1 100mM</p> <p>Methanol n/a</p> <p>MOPS pH 7.2 200mM</p> <p>MOPS pH 7.2 200mM</p> <p>M-PER Reagent 1.2</p> <p>n-Acetylglucosamine 0</p>	<p>2-D sample buffer¹ n/a</p> <p>2-Mercaptoethanol 1mM</p> <p>ACES, pH 7.8 10mM</p> <p>Acetone 10%</p> <p>Acetonitrile 10%</p> <p>Ammonium sulfate 10mM</p> <p>Aprotinin 10µg/mL</p> <p>Ascorbic acid 50mM</p> <p>Asparagine 100mM</p> <p>Bicline 100mM</p> <p>Bis-Tris pH 6.5 100mM</p> <p>Borate (50mM) undiluted</p> <p>pH 8.5</p> <p>B-PER Reagent 1.2</p> <p>B-PER Reagent II 1.2</p> <p>B-PER Reagent PPS n/a</p> <p>Brij-35 0.062%</p> <p>Brij-56 0.031%</p> <p>Brij-58 0.016%</p> <p>Bromophenol blue (in 50mM NaOH)</p> <p>Calcium chloride 10mM</p> <p>Cesium bicarbonate 100mM</p> <p>Cetyltrimethylammonium chloride n/a</p> <p>CHAPS 5%</p> <p>CHAPS 5%</p> <p>CHES 100mM</p> <p>Cobalt chloride (in 50mM NaOH)</p> <p>CitAB n/a</p> <p>Cysteine 10mM</p> <p>Dithioerythritol (DTE) 10mM</p> <p>Dithiothreitol (DTT) 10mM</p> <p>DMF 10%</p> <p>DMSO 10%</p> <p>DTAB 10%</p> <p>EDTA 10%</p> <p>EPPS pH 8.0 100mM</p> <p>Ethanol 10%</p> <p>Ferric chloride (in 10mM NaOH)</p> <p>Ferric chloride 10mM</p> <p>Glucose 100mM</p> <p>Glutathione (reduced) n/a</p> <p>Glycerol (fresh) 10%</p> <p>Glycine-HCl pH 2.8 100mM</p> <p>Guandine-HCl 3.5M</p> <p>HEPES pH 7.5 100mM</p> <p>Hydrides (NaBH₄ or NaCNBH₃) n/a</p> <p>Hydrochloric acid (in 10mM NaOH)</p> <p>I-PER Reagent n/a</p> <p>Laemmli SDS n/a</p> <p>Laemmli SDS sample buffer¹ n/a</p> <p>Leupeptin 10µg/mL</p> <p>Mannitol n/a</p> <p>Melibiose 100mM</p> <p>MES pH 6.1 100mM</p> <p>Methanol 10%</p> <p>Modified Dulbecco's undiluted</p> <p>MOPS pH 7.2 100mM</p> <p>MOPS pH 7.2 100mM</p>	<p>2-D sample buffer¹ n/a</p> <p>2-Mercaptoethanol 1mM</p> <p>ACES, pH 7.8 10mM</p> <p>Acetone 10%</p> <p>Acetonitrile 10%</p> <p>Ammonium sulfate 1M</p> <p>Aprotinin 10µg/mL</p> <p>Ascorbic acid 50mM</p> <p>Asparagine 10mM</p> <p>Bicline n/a</p> <p>Bis-Tris pH 6.5 100mM</p> <p>Borate (50mM) undiluted</p> <p>pH 8.5</p> <p>B-PER Reagent 1.2</p> <p>B-PER Reagent II 1.2</p> <p>B-PER Reagent PPS n/a</p> <p>Brij-35 0.031%</p> <p>Brij-56 0.062%</p> <p>Brij-58 0.031%</p> <p>Bromophenol blue (in 50mM NaOH)</p> <p>Calcium chloride 10mM</p> <p>Cesium bicarbonate 100mM</p> <p>Cetyltrimethylammonium chloride n/a</p> <p>CHAPS 5%</p> <p>CHAPS 5%</p> <p>CHES 100mM</p> <p>Cobalt chloride (in 50mM NaOH)</p> <p>CitAB n/a</p> <p>Cysteine 10mM</p> <p>Dithioerythritol (DTE) 10mM</p> <p>Dithiothreitol (DTT) 10mM</p> <p>DMF 10%</p> <p>DMSO 10%</p> <p>DTAB 10%</p> <p>EDTA 10%</p> <p>EPPS pH 8.0 100mM</p> <p>Ethanol 10%</p> <p>Ferric chloride (in 10mM NaOH)</p> <p>Ferric chloride 10mM</p> <p>Glucose 100mM</p> <p>Glutathione (reduced) n/a</p> <p>Glycerol (fresh) 10%</p> <p>Glycine-HCl pH 2.8 100mM</p> <p>Guandine-HCl 3.5M</p> <p>HEPES pH 7.5 100mM</p> <p>Hydrides (NaBH₄ or NaCNBH₃) n/a</p> <p>Hydrochloric acid (in 10mM NaOH)</p> <p>I-PER Reagent n/a</p> <p>Laemmli SDS n/a</p> <p>Laemmli SDS sample buffer¹ n/a</p> <p>Leupeptin 10µg/mL</p> <p>Mannitol n/a</p> <p>Melibiose 100mM</p> <p>MES pH 6.1 100mM</p> <p>Methanol 10%</p> <p>Modified Dulbecco's undiluted</p> <p>MOPS pH 7.2 100mM</p> <p>MOPS pH 7.2 100mM</p>	<p>2-D sample buffer¹ n/a</p> <p>2-Mercaptoethanol 1mM</p> <p>ACES, pH 7.8 10mM</p> <p>Acetone 10%</p> <p>Acetonitrile 10%</p> <p>Ammonium sulfate 0</p> <p>Aprotinin 10µg/mL</p> <p>Ascorbic acid 1mM</p> <p>Asparagine 5mM</p> <p>Bicline n/a</p> <p>Bis-Tris pH 6.5 n/a</p> <p>Borate (50mM) pH 8.5 n/a</p> <p>B-PER Reagent n/a</p> <p>B-PER Reagent II n/a</p> <p>B-PER Reagent PPS n/a</p> <p>Brij-35 0.031%</p> <p>Brij-56 0.062%</p> <p>Brij-58 0.031%</p> <p>Bromophenol blue (in 50mM NaOH)</p> <p>Calcium chloride 10mM</p> <p>Cesium bicarbonate 50mM</p> <p>Cetyltrimethylammonium chloride n/a</p> <p>CHAPS 0.031%</p> <p>CHAPS 0.031%</p> <p>CHES n/a</p> <p>Cobalt chloride (in 50mM NaOH)</p> <p>CitAB n/a</p> <p>Cysteine 1mM</p> <p>Dithioerythritol (DTE) 0</p> <p>Dithiothreitol (DTT) 0</p> <p>DMF 0.25%</p> <p>DMSO 0.25%</p> <p>DTAB 0.25%</p> <p>EDTA 0.25%</p> <p>EPPS pH 8.0 0.01%</p> <p>Ethanol 0.1%</p> <p>Ferric chloride (in 10mM NaOH)</p> <p>Ferric chloride 0.1%</p> <p>Glucose 100mM</p> <p>Glutathione (reduced) n/a</p> <p>Glycerol (fresh) 10%</p> <p>Glycine-HCl pH 2.8 100mM</p> <p>Guandine-HCl 3.5M</p> <p>HEPES pH 7.5 100mM</p> <p>Hydrides (NaBH₄ or NaCNBH₃) n/a</p> <p>Hydrochloric acid (in 10mM NaOH)</p> <p>I-PER Reagent n/a</p> <p>Laemmli SDS n/a</p> <p>Laemmli SDS sample buffer¹ n/a</p> <p>Leupeptin 10µg/mL</p> <p>Mannitol n/a</p> <p>Melibiose 25mM</p> <p>MES pH 6.1 125mM</p> <p>Methanol n/a</p> <p>Modified Dulbecco's undiluted</p> <p>MOPS pH 7.2 n/a</p> <p>MOPS pH 7.2 n/a</p>

Concentrations listed refer to the actual concentration in the protein sample that is compatible with the assay.

n/a Compound was not tested in this assay.

0 Compound that is not compatible at the lowest concentration tested.

¹ Value when the 660nm Assay is run using the ionic detergent compatibility reagent (IDCR, Part No. 22663).

² Selected values for the regular BCA-RAC kit are given in parentheses in the column for the Microplate BCA-RAC.

³ Compound (buffer) whose formulation is described more fully in the following table:

Buffer	Formulation
2-D sample buffer	(8M urea, 4% CHAPS) or (7M urea, 2M thiourea, 4% CHAPS)
Laemmli SDS sample buffer	65mM Tris-HCl, 10% glycerol, 2% SDS, 0.025% bromophenol blue
MES-buffered saline pH 4.7	0.1M MES, 150mM NaCl pH 4.7
Modified Dulbecco's PBS	8mM sodium phosphate, 2mM potassium phosphate, 0.14M NaCl, 10mM KCl, pH 7.4
Na carb-bicarb pH 9.4	0.2M sodium carbonate-bicarbonate, pH 9.4
Na citrate-carbonate pH 9	0.0M sodium citrate, 0.1M sodium carbonate, pH 9
Na citrate-MOPS pH 7.5	0.0M sodium citrate, 0.1M MOPS, pH 7.5
Phosphate-buffered saline (PBS)	100mM sodium phosphate, 150mM NaCl, pH 7.2
RIPA Buffer	50mM Tris, 150mM NaCl, 0.5% DDC, 1% NP-40, 0.1% SDS, pH 8.0
Tris-buffered saline (TBS)	25mM Tris, 150mM NaCl, pH 7.6
Tris-glycine pH 8.0	25mM Tris, 192mM glycine, pH 8.0
Tris-glycine SDS pH 8.3	25mM Tris, 192mM glycine, 0.1% SDS, pH 8.3
Tris-HEPES-SDS	100mM Tris, 100mM HEPES, 3mM SDS

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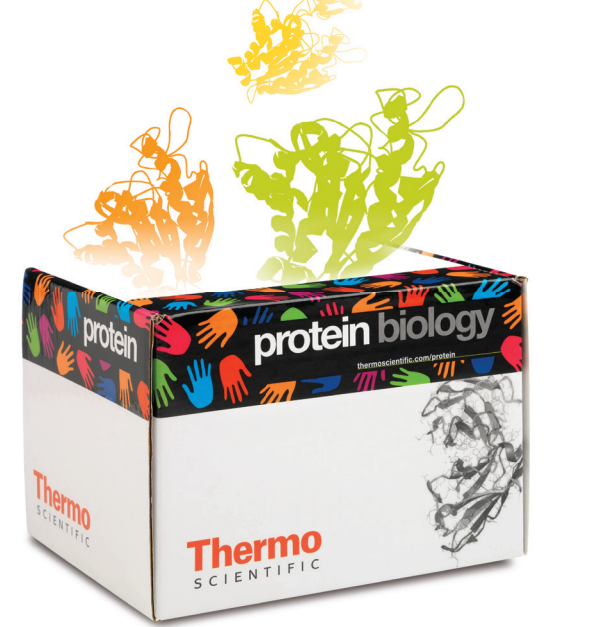
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