

FLAG Affinity Purification

FLAG® Immunoprecipitation Kit

**FLAGIPT-1** Features and Benefits 1 kit 367.05  
 -20-0°C

- Utilized highly specific ANTI-FLAG® M2 affinity gel
- No preliminary steps or calibrations
- 3XFLAG® peptide provides efficient and gentle elution through direct competition

1 kit sufficient for 50 reactions

**Components:**

- ANTI-FLAG M2 affinity gel, 1 mL
- Elution buffer, 30 mL
- FLAG-BAP™ control protein, 50 µg
- 3X FLAG peptide, 1 mg
- Lysis buffer, 50 mL
- 2X Sample buffer, 1.5 mL
- 10X wash buffer, 30 mL

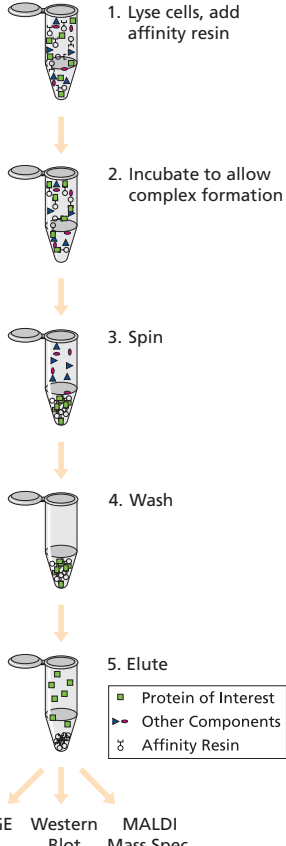


Figure 1. Affinity-based molecular pull-down technique.

FLAG® 96-well Immunoprecipitation Kit

**HT-COIP-1** The FLAG® 96-Well Immunoprecipitation Kit provides a unique method to validate protein-protein interactions in a convenient 96-well format. The kit contains the ANTI-FLAG® 96-well plate and all reagents necessary for capture and elution of FLAG fusion proteins. 1 kit 367.65  
 2-8°C

- Rapid: ELISA format allows analysis of protein-protein interactions in less than 4 hours
- High-throughput: 96-well plate configuration greatly increases throughput
- No antibody leaching: ANTI-FLAG® M2 antibody is covalently coupled to the surface of the well, therefore no interference from antibody on Western blots

**Features and Benefits**

- Rapid: ELISA format allows analysis of protein-protein interactions in less than 4 hours
- High-throughput: 96-well plate configuration greatly increases throughput
- No antibody leaching: ANTI-FLAG® M2 antibody is covalently coupled to the surface of the well, therefore no interference from antibody on Western blots

**Components:**

- ANTI-FLAG® High Sensivity, M2 coated 96-well plates, 1 ea
  - Amino-terminal FLAG-BAP fusion protein, 2.5 µg
  - 10x Wash buffer, 50 ml
  - Lysis buffer, 50 ml
  - 2x Sample buffer, 6 ml
  - SealPlate Film, 6 sheets
- FLAG products and/or their use are covered by one or more the the following Sigma-Aldrich owned patents: US 4,703,004; US 4,851,351; US 5,011,912; CA 1307752; EP 150126; EP 335899; JP 198150 and JP 2665359.  
 R: 22-36/37/38-41 S: 26-36

FLAG® Peptide

**F 3290** (Asp-Tyr-Lys-Asp-Asp-Asp-Lys) 4 mg 86.35  
 2-8°C C<sub>41</sub>H<sub>60</sub>N<sub>10</sub>O<sub>20</sub> FW 1012 25 mg 408.05

- Lyophilized powder**
- Gently elutes FLAG fusion proteins from ANTI-FLAG® M1 and M2 affinity resins. Usual working concentration is 100 µg/ml. Will not elute 3X FLAG fusion proteins. For this application, use 3X FLAG Peptide, F 4799.

3X FLAG® Peptide

**F 4799** (Met-Asp-Tyr-Lys-Asp-His-Asp-Gly-Asp-Tyr-Lys-Asp-His-Asp-Ile-Asp-Tyr-Lys-Asp-Asp-Asp-Lys) 4 mg 174.75  
 2-8°C C<sub>120</sub>H<sub>169</sub>N<sub>31</sub>O<sub>49</sub>S FW 2861 25 mg 814.95

- Lyophilized powder**
- Gently elutes FLAG fusion proteins from the ANTI-FLAG® M2 affinity gels. 1X FLAG Peptide (F 3290) will not elute 3X FLAG fusion proteins from ANTI-FLAG affinity gels.

HIS-Select Affinity Purification

HIS-Select™ Nickel Affinity Gel

<b>P 6611</b>	Nickel Affinity Gel used for the purification of histidine-tagged proteins.	5 mL	57.80
<b>2-8°C</b>		25 mL	184.90
◆	The unique, non-charged, hydrophilic linkage of the NTA chelate group to the agarose ensures high selectivity for histidine-tagged proteins and reduced non-specific binding of other proteins. It's high selectivity allows for One-step purification. Affinity gel can be used in either denaturing or non-denaturing conditions.	100 mL	598.00
		500 mL	2620.00

Features and Benefits

- High selectivity for higher purity
- Unique non-charged hydrophilic linkage reduced non-specific binding
- Binding capacity for histidine-tagged protein is greater than 15 mg/ml
- Binding under denaturing or non-denaturing conditions
- One-step purification

50% suspension in 30% ethanol  
It is also available with the EZview™ technology (Product Code E3528).

Matrix . . . . . 6% Beaded Agarose  
binding capacity (protein) . . . . . >15 mg/mL (with an approx. 30 kDa protein)

Licensed for use under patent U.S. 4,569,794 with additional patents pending by Sigma.

R: 10-36/37/38 S: 16-26-36

HIS-Select™ HF Nickel Affinity Gel

<b>H 0537</b>	Nickel Affinity Gel for the purification of histidine tagged proteins. HIS-Select™ High Flow (HF) brings the superior selectivity of HIS-Select technology to a highly cross-linked agarose for higher flow rates and mechanical stability under pressure. HIS-Select HF is designed for production scale purification and FPLC™ applications. As with other HIS-Select products, the non-charged, hydrophilic linkage of the nickel-nitrilotriacetic acid (Ni-NTA) chelate group to the agarose ensures true one-step purification.	10 mL	102.00
<b>2-8°C</b>		25 mL	234.60
◆		100 mL	780.30
		500 mL	3290.00

NEW

Features and Benefits

- High selectivity for higher purity
- Unique non-charged hydrophilic linkage reduced non-specific binding
- Binding Capacity for histidine-tagged protein is greater than 15 mg/ml
- Highly cross-linked agarose matrix allows for purification under higher pressures
- Binding under denaturing or non-denaturing conditions
- One-step purification

50% suspension in 30% ethanol  
Matrix . . . . . Highly cross-linked 6% Beaded Agarose  
binding capacity (protein) . . . . . 15 mg/mL (with an approx. 30 kDa protein)

Licensed for use under U.S. patent 4,569,794 with additional patents pending by Sigma.

R: 11-36/37/38 S: 9-16-26-29-33-36

HIS-Select Cartridge

<b>H 8286</b>	The HIS-Select Cartridge is a prepacked ready to use cartridge (column) that contains 1.25 ml of HIS-Select Nickel Affinity Gel. This cartridge's luer lock fittings make it suitable for Low Pressure Liquid Chromatography Systems (LPLC), Medium Pressure Liquid Chromatography Systems (MPLC), and for use with syringes.	5 each	91.80
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WET ICE

NEW

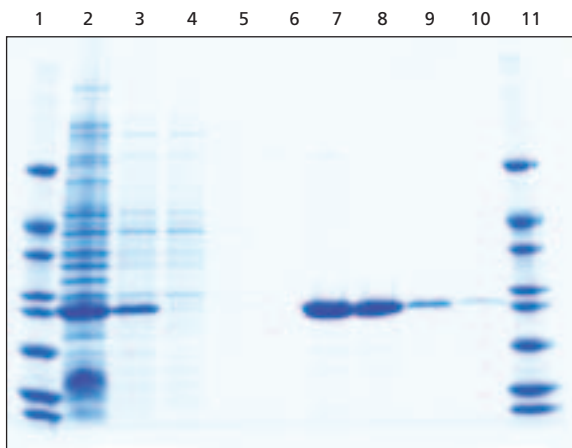
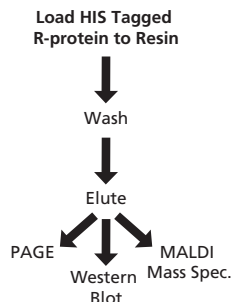
HIS-Select Nickel Affinity Gel is an immobilized metal-ion affinity chromatography (IMAC) product. The HIS-Select Nickel Affinity gel is a proprietary, patent pending, quadridentate chelate on beaded agarose charged with nickel that is designed to specifically bind histidine containing proteins. The matrix for this affinity gel is 6% beaded agarose. HIS-Select Nickel Affinity Gel is selective for recombinant proteins with histidine tags and exhibits low non-specific binding of other proteins.

The HIS-Select Cartridge has a storage buffer that contains 30% ethanol.

binding capacity . . . ≥15 mg/mL (as determined with an approx. 30 kDa histidine-tagged protein)

Licensed for use under patent U.S. 4,569,794 with additional patents pending by Sigma.

R: 10-36/37/38 S: 16-26-36



- |  |   |
|--|---|
| 1 Low Range Sigma Marker (M 3913)      | 7 Elute 2                                     |
| 2 Whole Cell Extract in Sample Buffer  | 8 Elute 3                                     |
| 3 Whole Cell Extract with CellLytic B™ | 9 Elute 4                                     |
| 4 Flow Through                         | 10 Elute 5                                    |
| 5 Wash                                 | 11 Low Molecular Weight Sigma Marker (M 3913) |
| 6 Elute 1                              |   |

Figure 1. One-Step Purification

## HIS-Select Affinity Purification

## HIS-Select Spin Columns

**H 7787** Pre-packed and ready-to-use HIS-Select Spin Columns allow for fast and consistent purification of small-scale crude cell extracts containing histidine-tagged proteins. The Spin Columns contain a matrix of silica particles and uses the HIS-Select patent-pending non-charged, hydrophilic nickel chelating linkage chemistry. HIS-Select Spin Columns are highly selective for histidine tagged proteins and exhibits very low non-specific binding of other proteins. These convenient Spin Columns allow for fast, one-step purification for the isolation of at least 150 µg of histidine-tagged protein in less than 15 minutes.

**Features and Benefits**

- High Selectivity for higher purity
  - Convenient and Ready-to-Use
  - Consistent protein binding each use
  - Compatible with CellLytic™ cell lysis reagents
- Kit contains 50 HIS-Select Spin Columns and 150 collection tubes

## EZview™ Red HIS-Select™ HC Nickel Affinity Gel

**E 3528** When performing small scale affinity capture, such as immunoprecipitation, the affinity matrix is difficult to see in the microcentrifuge tubes. Accidental aspiration of the resin leads to quantitative variability in results. The EZview™ Red Affinity Gels greatly reduces the risk of pellet loss. EZview™ resins perform as well as conventional non-colored affinity gels, but allow the user to easily differentiate pellet from supernatant. This correlates to more accurate data because less protein is lost.

Small-scale affinity capture of fusion proteins with histidine containing tags.

**Features and Benefits**

- Increased visibility - Red color reduces risk of incidental aspiration
  - Improved recovery of target protein by reduced accidental loss
  - Higher reproducibility - More consistent yields
- 1:1 (v/v) suspension in PBS containing 50% glycerol and 15 ppm Kathon  
binding capacity (protein). . . . . ≥15 mg/mL  
Licensed for use under patent U.S. 4,569,794 with additional patents pending by Sigma.

## HIS-Select™ Nickel Coated HC 96-well plate

**S 5563** HIS-Select™ 96-well clear high capacity coated plates are coated with a proprietary high-density nickel chelate matrix. This patent pending high capacity coating provides a greater per-well binding capacity than any other commercial histidine binding plate and can be used in a variety of applications including in-well protein quantification, high throughput purification, and post-capture analysis such as MALDI.

**Features and Benefits**

- Pre-treated to reduce non-specific binding, Sigma's HIS-Select high capacity coated multiwell plates provide a binding capacity greater than 4 µg/well.
  - Manufactured under ISO 9002 in Sigma's GMP facility.
- polystyrene  
flat bottom  
96-well, clear  
Licensed for use under patent U.S. 4,569,794 with additional patents pending by Sigma.

## Additional Affinity Purification

## EZview™ Red Protein A Affinity Gel

**P 6486** When performing small scale affinity capture, such as immunoprecipitation, the affinity matrix is difficult to see in the microcentrifuge tubes. Accidental aspiration of the resin leads to quantitative variability in results. The EZview™ Red Affinity Gels greatly reduces the risk of pellet loss. EZview™ resins perform as well as conventional non-colored affinity gels, but allow the user to easily differentiate pellet from supernatant. This correlates to more accurate data because less protein is lost.

Immunoprecipitation of proteins

**Features and Benefits**

- Increased visibility - Red color reduces risk of incidental aspiration
  - Improved recovery of target protein reduces the need to repeat due to accidental loss
  - Higher reproducibility - More consistent yields
- 1:1 (v/v) suspension in PBS containing 50% glycerol and 15 ppm Kathon  
binding capacity (rabbit IgG). . . . . ≥4 mg  
S: 23-24/25

## Protein A coated HS 96-well plates

**S 1938** Protein A is a 42 kDa polypeptide that binds specifically with the Fc region of the IgG of many mammalian species. The IgG may be applied directly to the surface or captured as an antibody/antigen complex. Once captured, the antibody's antigen binding site is orientated away from the surface. Protein A coated high sensitivity multiwell plates are coated with a recombinant form of Protein A which binds specifically to the Fc region of IgG of many species including human and mouse. Once captured, the antibody's antigen binding site is oriented away from the surface allowing for better performance in downstream applications. Pre-blocked for immediate use and manufactured under ISO 9002 in Sigma's GMP facility, Protein A high sensitivity coated multiwell plates utilize a flat bottom, polystyrene baseplate.

96-well, clear

## EZview™ Red Protein G Affinity Gel

**E 3403** When performing small scale affinity capture, such as immunoprecipitation, the affinity matrix is difficult to see in the microcentrifuge tubes. Accidental aspiration of the resin leads to quantitative variability in results. The EZview™ Red Affinity Gels greatly reduces the risk of pellet loss. EZview™ resins perform as well as conventional non-colored affinity gels, but allow the user to easily differentiate pellet from supernatant. This correlates to more accurate data because less protein is lost.

Immunoprecipitation of epitope tagged fusion proteins.

**Features and Benefits**

- Increased visibility - Red color reduces risk of incidental aspiration
  - Improved recovery of target protein reduces the need to repeat due to accidental loss
  - Higher reproducibility - More consistent yields
- 1:1 (v/v) suspension in PBS containing 50% glycerol and 15 ppm Kathon  
binding capacity (rabbit IgG). . . . . ≥8 mg/mL

## Additional Affinity Purification

## Protein G coated HS 96-well plates

**S 2063** Protein G specifically binds the Fc region of the IgG of many mammalian species. Protein G HS 96-well plates are coated with a genetically engineered, truncated form of Protein G that reduces non-specific binding. The IgG may be applied directly to the surface or captured as an antibody/antigen complex. Once captured, the antibody's antigen binding site is oriented away from the surface to ensure optimal capture.

polystyrene  
flat bottom  
96-well, clear

## EZview™ Red Glutathione Affinity Gel

**E 2404** When performing small scale affinity capture, such as immunoprecipitation, the affinity matrix is difficult to see in the microcentrifuge tubes. Accidental aspiration of the resin leads to quantitative variability in results. The EZview™ Red Affinity Gels greatly reduces the risk of pellet loss. EZview™ resins perform as well as conventional non-colored affinity gels, but allow the user to easily differentiate pellet from supernatant. This correlates to more accurate data because less protein is lost. Used for affinity capture, molecular pull-down, or IP of proteins containing glutathione binding sequences, such as glutathione S-transferase, glutathione peroxidase and glyoxalase I.

## Features and Benefits

- Increased visibility - Red color reduces risk of incidental aspiration
  - Improved recovery of target protein, reduced accidental loss
  - Higher reproducibility - More consistent yields
- 1:1 (v/v) suspension in PBS containing 50% glycerol and 15 ppm Kathon  
S: 23-24/25

## Influenza Hemagglutinin (HA) Peptide

**I 2149** (HA tag peptide; HA1 fragment 98-106; Tyr-Pro-Tyr-Asp-Val-Pro-Asp-Tyr-Ala)  
CAS No. 92000-76-5  
C<sub>53</sub>H<sub>67</sub>N<sub>9</sub>O<sub>17</sub> FW 1102  
**minimum 97% (HPLC)**

Useful for displacement of HA-tagged fusion proteins from HA antibody in immunoassays. The successful inhibition of antibody binding by HA peptide demonstrates binding is specific.

## References

Niman, H., et al., Generation of protein-reactive antibodies by short peptides is an event of high frequency: Implications for the structural basis of immune recognition. *Proc. Nat. Acad. Sci. USA* **80**, 4949-4953 (1983)

## Monoclonal Anti-HA Agarose

**A 2095** antibody produced in mouse Clone HA-7, Purified immunoglobulin, PBS suspension 1 mL 248.00

**2-8°C**  
WET ICE Immunogen: A synthetic peptide corresponding to amino acid residues 98-106 (YPYDVPDYA) of human Influenza virus hemagglutinin (HA) known as HA-tag, conjugated to KLH. The product is reactive with N- or C-terminal HA-tagged fusion proteins expressed in *E. coli* or in mammalian cells. Monoclonal Anti-HA-Agarose recognizes native and denatured-reduced fusion proteins expressed in *E. coli* or in mammalian cells. Specific antibody concentration is 2.0-2.4 mg/ml settled resin. Suspension in 0.01 M phosphate buffered saline, containing 15 mM sodium azide. Prepared using cyanogen bromide-activated agarose. Clone HA-7  
Isotype: . . . . . IgG1

## Anti-c-Myc Agarose Affinity Gel

**A 7470** Anti-c-Myc recognizes an epitope located on c-Myc tagged fusion proteins. Used for the immunoprecipitation or immunoaffinity purification of fusion proteins. Specificity: N-terminal or C-terminal c-myc (Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu) tagged fusion proteins. Suspension of beaded agarose (1:1 v/v) in 0.01 M phosphate buffered saline, containing 15 mM sodium azide. . . . . 2 nmol/mL

## Anti-c-Myc Coated HS 96-well Plates

**P 2241** The c-Myc epitope tag is well known and highly immunoreactive. The plates allow capture and purification of c-myc tagged fusion proteins. Detection levels were determined to be <1 ng/well. Suitable for screening for expression, study of protein:protein interactions and ELISA assay. Specificity: N-terminal or C-terminal c-Myc (Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu) tagged fusion proteins. clear polystyrene flat-bottomed 96-well plates

## c-Myc

**M 2435** (Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu) Lyophilized powder 4 mg 94.25 25 mg 443.30  
**2-20°C**  
Amino acid sequence corresponds to amino acids 410-419 of the C-terminal of human c-myc. Useful for displacement of c-Myc-tagged fusion proteins bound to anti-c-Myc antibodies in immunoassays. The successful inhibition of antibody binding by c-Myc peptide demonstrates binding is specific. mol wt 1203.3 Da

## Anti-Thioredoxin-Agarose

**A 2582** antibody produced in rabbit IgG fraction of antiserum, PBS suspension 1 mL 371.60

**2-8°C**  
WET ICE Immunogen: Recombinant *E. coli* thioredoxin. The antibody is specific for natural *E. coli* and recombinant thioredoxin. It may be used to identify and purify the expression of thioredoxin fusion proteins. Suspension in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide. Prepared using antibody coupled to cyanogen bromide activated agarose at 5 mg antibody per ml resin volume.

## Additional Affinity Purification

## Anti-V5 Agarose Affinity Gel

**A 7345 antibody produced in mouse** 1 mL 236.20  
 [2-8°C] **Purified immunoglobulin, Clone V5-10**  
 ◆ Suitable for affinity purification and immunoprecipitation of V5 tagged fusion proteins.  
 WET ICE Recognizes V5 Tag (GKPIPPLLGLDST) fusion proteins  
 Suspension of beaded agarose (1:1 v/v) in 0.01 M phosphate buffered saline, containing 15 mM sodium azide.  
 Capacity (V5 fusion protein). . . . . 2.5 nmol/mL

## V5

**V 7754 (CGKPIPPLLGLDST)** 4 mg 138.60  
 [-20°C] **minimum97% (HPLC), Lyophilized powder** 25 mg 648.35  
 ◆ The amino acid sequence corresponds to amino acids 95-108 of non-structural protein V and to RNA polymerase  $\alpha$  subunit (P protein), of Paramyxovirus SV5 with an N-terminal cysteine. The peptide is useful for displacement of V5-tagged fusion proteins from anti-V5 antibody in immunoassays. The successful inhibition of antibody binding of V5 peptide demonstrates binding is specific.  
 calculated mol wt 1524.8 Da

## Protein Quantitation

## Bicinchoninic Acid Kit for Protein Determination

**BCA-1** (BCA protein assay) 1 kit 147.45  
 [2-8°C] **for 200-1000  $\mu\text{g/ml}$  protein**  
 Proteins reduce alkaline Cu(II) to Cu(I) in a concentration-dependent manner. Bicinchoninic acid is a highly specific chromogenic reagent for Cu(I), forming a purple complex with an absorbance maximum at 562 nm. The absorbance is directly proportional to protein concentration. This is an alternative to the Folin-Ciocalteu reagent for protein determination.

## Features and Benefits

- Simple, sensitive colorimetric assay for proteins
- Two stable reagents are used for the working solution
- Faster and easier than the Lowry protein assay
- Compatible with many detergents both ionic and nonionic
- Less variation between different proteins than the Bradford dye-binding assay
- Adaptable for use in microwell plates sufficient for approx. 500 assays (tests + controls)

## Components:

Bicinchoninic Acid Solution, 1 L  
 4%(w/v)  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  Solution, 25 mL  
 Protein Standard Solution, 5  $\times$  1 mL

## References

1. Lowry, O.H., et al., Protein measurement with the Folin phenol reagent *J. Biol. Chem.* **193**, 265-275 (1951)
2. Smith, P.K., et al., Measurement of protein using bicinchoninic acid. *Anal. Biochem.* **150**, 76-85 (1985)
3. Brown, R.E., et al., Protein measurement using bicinchoninic acid: elimination of interfering substances. *Anal. Biochem.* **180**, 136-139 (1989)
4. Wieheman, K., Braun, R. and Fitzpatrick, J., Investigation of the bicinchoninic acid protein assay: identification of the groups responsible for color formation. *Anal. Biochem.* **175**, 231-237 (1988)  
 R: 36/37/38 S: 26-36

## Bicinchoninic Acid solution

**B 9643** (BCA solution) 1 L 139.90  
 [RT] **liquid**  
 Also available as part of the Bicinchoninic Acid Kit for Protein Determination (BCA-1).  
 suitable for determination of protein concentration  
 Color. . . . . clear, colorless  
**References**  
 Smith, P.K., et al., Measurement of protein using bicinchoninic acid. *Anal. Biochem.* **150**, 76-85 (1985)  
 R: 36/37/38 S: 26-36

## QuantiPro™ BCA Assay Kit

**QP-BCA** **for 0.5-30  $\mu\text{g/ml}$  protein** 1 kit 163.85  
 [RT] Based on the alkaline reduction of Cu(II) to Cu(I) by proteins, and the formation of a bicinchoninic acid:Cu(I) complex having an absorbance maximum at 562 nm  
 Can be used to measure very dilute protein concentrations in very small sample volumes. Accurately measures protein concentrations from 0.5 to 30  $\mu\text{g/mL}$  in tube assays and 1 to 20  $\mu\text{g/mL}$  in 96- or 384-well plate assays.

NEW

## Features and Benefits

- Accurate across a broad range of protein concentrations
- High sensitivity; linear response from 0.5 to 30  $\mu\text{g/ml}$  of protein
- Stable color complex
- Reduced susceptibility to detergents

## Components:

Quantipro buffer QA, 250 mL  
 Quantipro BCA QB, 250 mL  
 Protein Standard Solution: 1.0 mg/ml bovine serum albumin in 0.15 M NaCl with 0.05% sodium azide (flame-sealed glass ampules), 10 $\times$ 1 mL  
 4% Copper(II) sulfate pentahydrate solution, 12 mL

## References

1. Wieheman, K., Investigation of the bicinchoninic acid protein assay: identification of the groups responsible for color formation. *Anal. Biochem.* **175**, 231 (1988)
2. Brown, R.E., Protein measurement using bicinchoninic acid: elimination of interfering substances *Anal. Biochem.* **180**, 136 (1989)  
 R: 60-22-37/38-41-43-63 S: 53-26-27-36/37/39-45

## Bradford Reagent

**B 6916** (Coomassie® dye binding protein assay, 500 mL 35.50  
 [2-8°C] Protein dye reagent)

◆ for 1-1,400  $\mu\text{g/ml}$  protein

This protein assay is based on complexing of proteins with Brilliant Blue G. The protein sample is mixed with the reagent and then read at 595 nm after a short incubation at room temperature.

## Features and Benefits

- The reagent is ready to use. No mixing or dilution required.
  - Color development is rapid. Only a five minute incubation and then the sample is read a 595 nm.
  - Reducing sugars and reducing substances along with thiols do not interfere with this reagent.
  - Reagent is suitable for micro (1-10  $\mu\text{g/ml}$ ) and standard (50-1400  $\mu\text{g/ml}$ ) assays.
  - Can be used in microwell plate assays.
  - Inexpensive assay.
- Data sheet included with each bottle.  
 Coomassie is a trademark of ICI Americas