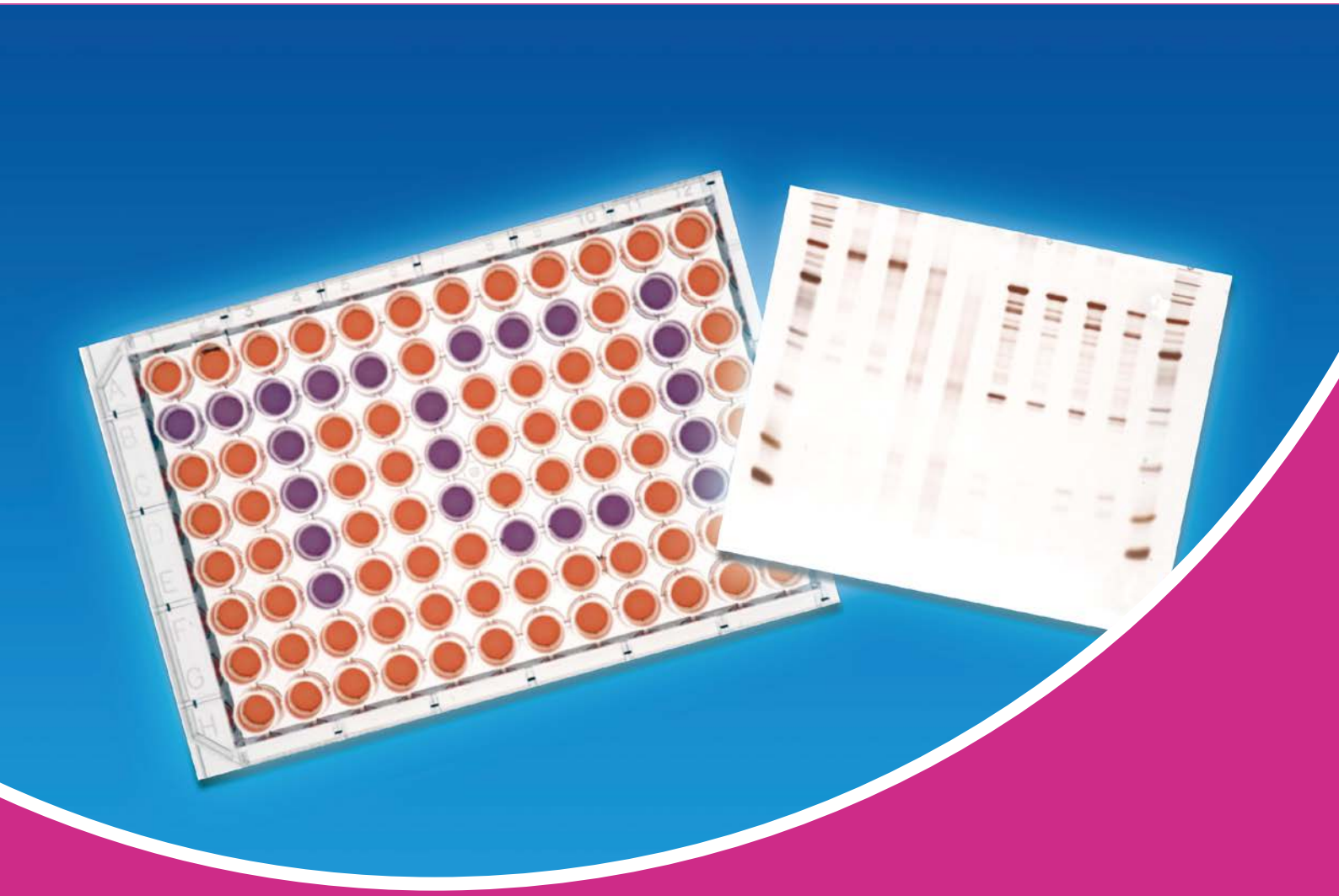
LIFE SCIENCE

Protein and Biological Sample Preparation Reagents



Surfactants/Detergents

Surfactants are amphiphilic compounds containing both hydrophobic and hydrophilic groups and thereby are soluble in both organic solvents and water. Owing to the characteristic behavior of surfactants to orient at surfaces and form micelles by reducing the surface tension, they play an important role in many practical applications including the solubilization of membrane proteins, the decrease of the nonspecific adsorption of the material to the container surface etc. in the area of biochemistry. Surfactants are classified as ionic or nonionic depending on the formal charge on their hydrophilic head. Ionic surfactants can further be cationic, anionic or zwitter-ionic on the basis of the type of the charge present. The selection of surfactants in different fields is decided according to their particular usage.

Anionic Surfactants

Lithium Dodecyl Sulfate (=LDS)	5g / 25g	[L0254]
Sodium Dodecyl Sulfate (=SDS)	25g / 500g	[S0588]
Tris Dodecyl Sulfate	250mg / 1g	[T3071]
Sodium Deoxycholate	25g	[D1820]
Sodium Cholate	5g / 25g	[S0596]
Sodium *N*-Lauroylsarcosinate Hydrate	5g / 25g	[S0597]

Amphoteric Surfactants

Lauryl Sulfobetaine	5g / 25g	[D3860]
Palmityl Sulfobetaine	5g / 25g	[H1283]
Myristyl Sulfobetaine	5g / 25g	[T2653]
Caprylyl Sulfobetaine	5g / 25g	[D4246]
***n*-Octyl Sulfobetaine**	5g	[D4247]

Nonionic Surfactants

TRITON™ X-100 (n=approx. 10)	5g / 25g	[P1775]
Polyethylene Glycol Monocetyl Ether (n=approx. 23)	5g / 25g	[P1776]
Polyethylene Glycol Monododecyl Ether (n=approx. 25)	5g / 25g	[P1777]
Tween 20 (=Polyoxyethylene Sorbitan Monolaurate)	5g / 25g	[T2530]
Tween 40 (=Polyoxyethylene Sorbitan Monopalmitate)	5g / 25g	[T2531]
Tween 60 (=Polyoxyethylene Sorbitan Monostearate)	5g / 25g	[T2532]
Tween 80 (=Polyoxyethylene Sorbitan Monooleate)	5g / 25g	[T2533]
Tween 85 (=Polyoxyethylene Sorbitan Trioleate)	5g / 25g	[T2534]
***n*-Octyl-β-D-Glucopyranoside**	1g	[O0355]

Non-Detergent Sulfobetaines (NDSB)

Non-detergent sulfobetaines (NDSB) are amphiphilic small compounds containing both a cationic and anionic component which do not form micelles because of their small hydrophobic moiety. NDSBs solubilize proteins under mild conditions and can prevent protein denaturation by heat or acid, inhibit protein aggregation, acceleration protein refolding, and aid membrane protein extraction.

NDSB 211	1g / 5g	[H1399]
NDSB 201	5g / 25g	[S0813]
NDSB 256-4T	1g	[B4030]

Please inquire for pricing and availability of listed products to our local sales representatives.

Protease Inhibitors

Proteolysis is one of the major problems during protein extraction as they result in decreased yields. The addition of inhibitors helps prevent proteolysis and improves recovery of the desired protein. Inhibitors are also used during immunoprecipitation to prevent decomposition of antigens or antibodies by proteolytic impurities.

Cysteine Protease Inhibitors

2-Iodoacetamide	5g [I0741]
E-64d	5mg / 25mg [E1337]

Serine Protease Inhibitors

AEBSF (=4-(2-Aminoethyl)benzenesulfonyl Fluoride Hydrochloride)	100mg / 1g [A2215]
Benzamidine Hydrochloride	5g [B3379]
Benzylsulfonyl Fluoride	5g / 25g [B3473]

Metalloprotease Inhibitors

EDTA 2Na Dihydrate	5g / 25g [D3789]
EDTA 3Na Hydrate	5g / 25g [T2599]
EGTA	5g / 25g [E0805]
1,10-Phenanthroline Monohydrate	5g [P1826]

Protein Denaturation Reagents

Proteins fold into higher-order structures due to interactions such as hydrogen bonding, ionic interactions, and Van der Waals forces. Heat, acids and alkalis can change protein conformation and denature proteins. Protein extraction and analysis require protein denaturation, necessitating the use of urea and guanidine, which are chaotropic agents that disrupt the hydrogen bonding network.

Guanidine Hydrochloride	25g / 100g / 500g [G0197]
Guanidine Thiocyanate	5g / 25g [G0360]
Thiourea	5g / 25g [T2835]
Urea	5g / 25g [U0077]

Nucleic Acid Removing Agents for Protein Sample Clarification

The process of nucleic acid removal may be effective in the purification of proteins. That is because the nucleic acid exhibits viscosity and the protein and the nucleic acid are likely to form a complex. The way to remove nucleic acid is absorption the nucleic acid to a basic water-soluble polymer or separation by binding and precipitating nucleic acid with nucleic acid removing agent such as streptomycin sulfate.

Polyethyleneimine (*ca.* 30% in Water)	25g / 100g [P1921]
Streptomycin Sulfate	5g / 25g [S0834]

Preservatives and Disinfectants

Research in the life sciences requires the analysis of biological samples. Microorganisms can easily grow in these samples, and also in the buffers and reagents used for biological analysis. Therefore, preservatives are frequently added to samples and buffers to prevent the growth of microorganisms.

Amprolium Hydrochloride	5g / 25g	[A2572]
2-*n*-Octyl-4-isothiazolin-3-one	1g	[O0378]
Dimetridazole	5g / 25g	[D4081]
2-Chloroacetamide	5g / 25g	[C2536]
5-Bromo-5-nitro-1,3-dioxane	5g	[B3769]
1,2-Benzisothiazol-3(2*H*)-one	5g	[B3767]
Sorbic Acid Potassium Salt	5g / 25g	[P1954]
Sorbic Acid	5g / 25g	[S0856]
1,3-Butanediol	5g / 25g	[B3770]
2-Phenoxyethanol	5g / 25g	[P1953]
2-Hydroxybenzoic Acid	5g / 25g	[H1342]
Benzoic Acid Sodium Salt	5g / 25g	[S0855]
Benzylparaben	5g / 25g	[B3768]
Isobutylparaben	5g / 25g	[I0816]
Butylparaben	5g / 25g	[B3771]
Isopropylparaben	5g / 25g	[I0817]
Propylparaben	5g / 25g	[P1955]
Ethylparaben	5g / 25g	[E0884]
Methylparaben	5g / 25g	[M2206]

Protein Determination Reagents

The determination of protein concentration is essential for biochemical research. The following two products are supplied as a ready-to-use solution for quantitative protein determination.

Reagent for Pyrogallol Red-Molybdate Protein Assay

Pyrogallol Red (Ready-to-use Solution) [for Protein determination] 100mL [P2575]

This product is supplied as a ready-to-use solution for protein determination based on the pyrogallol red-molybdate complex. When the dye binds proteins, the absorption maximum of the dye shifts from 480 nm to 600 nm in a linear manner with an increase in the quantity of the protein. It stains cuvettes very little, thus it can be washed with water alone after use.

Application

1. Prepare standard protein solutions with a series of dilutions.
2. Mix **P2575** with unknown protein samples, standard protein solutions and distilled water according to Table 1.
3. Incubate for 30 minutes at room temperature.
4. Measure absorbance at 600 nm.
5. Prepare a standard curve by plotting the absorbance data measured in #4) after subtracting from blank absorbance (distilled water), and calculate the amount of protein in test samples.

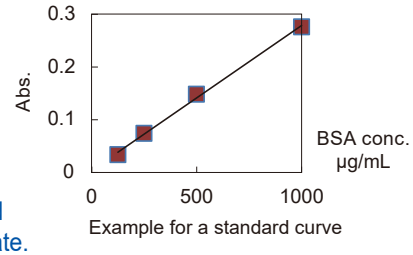
Table 1 : Volume for test tube or micro plate assay

Assay	test tube	micro plate
Measurement range	0.1 -1.0 mg/mL	0.1 -1.0 mg/mL
Sample solution or *protein standard	50 µL	10 µL
P2575	1 mL	200 µL

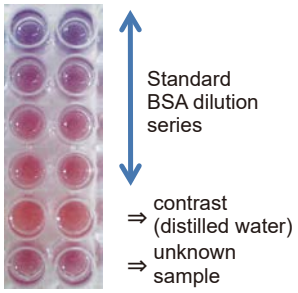
*This product requires the standard protein solution (such as BSA).

Example for use: in a microplate

1. Prepare four dilution series of standard protein solutions from the concentration at 1000 mg/mL by doubling dilution.
2. Mix 200 µL of **P2575** with 10 µL each of a protein sample at an unknown concentration, the standard protein solution and distilled water in a 96 microplate.
3. Incubate for 30 minutes at room temperature, measure absorbance at 600 nm, and prepare a standard curve.



Example for a standard curve



Example for a reaction

Compatible substance concentrations in protein sample of P2575

Substances at the following concentrations in the sample solutions do not affect the reaction results.

Buffers		Chelating Agents		Solvents	
Substance	Conc.	Substance	Conc.	Substance	Conc.
Glycine	100 mM	EDTA	100 mM	Acetone	10 %
Tris	2 M	EGTA	10 mM	DMSO	10 %
HCl	200 mM	Sodium citrate	200 mM	Ethanol	10 %
HEPES	100 mM			Methanol	10 %
MES	100 mM	**Salts**		Glycerol	10 %
MOPS	100 mM	Substance	Conc.		
PIPES	100 mM	$(NH_4)_2SO_4$	1 M	**Denaturants**	
Tricine	100 mM	KCl	1 M	Substance	Conc.
Imidazole	200 mM	$MgCl_2$	50 mM	DTT	100 mM
Glucose	1 M	$CaCl_2$	10 mM	Glutathione	1 mg / mL
Sucrose	25 %	$NiCl_2$	10 mM	2-Mercaptoethanol	1 M
Fructose	1 M	$ZnCl_2$	10 mM	Guanidine-HCl	1 M
		NaCl	2 M	Urea	3 M
		NaOH	100 mM		
		NaH_2PO_4	500 mM	**Detergents**	
		NaN_3	0.50 %	Substance	Conc.
				SDS	0.10 %
				Triton X-100	0.10 %
				Tween-20	0.10 %

P2575 requires the protein standard solution **[T3796]**.

Reagent for Bradford Assay

Bradford Assay Solution (Ready-to-use Solution) [for Protein determination] 500mL [B5702]

This product is supplied as a ready-to-use solution for protein assay based on the method of Bradford. This product contains Coomassie Brilliant Blue G-250 (CBB G-250). When the dye containing CBB G-250 binds proteins, the absorption maximum of the dye shifts from 465 to 595 nm linearly with the quantity of the protein. Absorbance can be measured only 5 minutes after the reaction starts. Low concentration of protein (1.0 - 25 µg/mL) can be measured.

Application

1. Prepare standard protein solutions with a series of dilutions.
2. Mix **B5702** with unknown protein samples, standard protein solutions and distilled water according to Table 2.
3. Incubate for 5 minutes at room temperature.
4. Measure absorbance at 600 nm.
5. Prepare a standard curve by plotting the absorbance data measured in #4) after subtracting from blank absorbance (distilled water), and calculate the amount of protein in test samples.

Table 1 : Volume for test tube or micro plate assay

Assay	test tube	micro plate	micro assay
Measurement range	0.1 -1.0 mg/mL	0.1 -1.0 mg/mL	0.1 - 25 µg/mL
Sample solution or *protein standard	20 µL	4 µL	500 µL
B5702	1 mL	200 µL	500 µL

*This product requires the standard protein solution (such as BSA).

Compatible substance concentrations in protein sample of B5702

Substances at the following concentrations in the sample solutions do not affect the reaction results.

Buffers		Salts		Solvents		Denaturants	
Substance	Conc.	Substance	Conc.	Substance	Conc.	Substance	Conc.
Glycine	100 mM	$(NH_4)_2SO_4$	1 M	Acetone	10 %	DTT	100 mM
Tris	2 M	KCl	1 M	DMSO	10 %	Glutathione	1 mg / mL
HCl	100 mM	$MgCl_2$	50 mM	Ethanol	10 %	2-Mercaptoethanol	1 M
HEPES	100 mM	$CaCl_2$	10 mM	Methanol	10 %	Guanidine-HCl	1 M
MES	100 mM	$NiCl_2$	10 mM	Glycerol	10 %	Urea	3 M
MOPS	100 mM	$ZnCl_2$	10 mM				
PIPES	100 mM	NaCl	2 M	**Detergents**		**Chelating Agents**	
Glucose	1 M	NaOH	100 mM	Substance	Conc.	Substance	Conc.
Sucrose	25 %	NaH_2PO_4	500 mM	SDS	0.05 %	EDTA	100 mM
Fructose	1 M	NaN_3	0.50 %	Triton X-100	0.10 %	EGTA	10 mM
				Tween-20	0.10 %	Sodium citrate	200 mM

B5702 requires the protein standard solution **[T3796]**.

Related Product

Standard Solution of Albumin from Bovine Serum 5mL **[T3796]**

Reagent for Bicinchoninic Acid (BCA) Assay

Bicinchoninic Acid Disodium Salt [for Protein Research] 5g **[B5838]**

Electrophoresis Reagents

Electrophoresis is a technique which separates charged biomolecules based on the rate at which they migrate in an applied electrical field. The following products are used in the Laemmli method, reagents widely used in protein staining and other related reagents.

Reagents for Gel Preparation, Buffer Preparation, etc.

2X SDS-PAGE Sample Buffer (2-Mercaptoethanol free)	25mL [B5834]
4X SDS-PAGE Sample Buffer (2-Mercaptoethanol free)	20mL [B6140]
6X Sample Buffer (2-Mercaptoethanol free)	10mL [B6105]
2X SDS-PAGE Sample Buffer Phenol Red (2-Mercaptoethanol free)	25mL [B6110]
30% Acrylamide / Bis-acrylamide (29:1)	250mL [A3217]
30% Acrylamide / Bis-acrylamide (37.5:1)	250mL [A3218]
Acrylamide Monomer	25g / 500g [A1132]
Ammonium Peroxodisulfate	5g / 25g [A2098]
Bromophenol Blue Sodium Salt (= BPB)	1g [B3195]
DL-Dithiothreitol (= DL-DTT)	1g / 5g [D3647]
Glycerol	1g [G0316]
Glycine	25g / 500g [G0317]
2-Mercaptoethanol	5g / 25g [M1948]
***N,N'*-Methylenebisacrylamide**	25g / 100g [M0506]
Sodium Dodecyl Sulfate (= SDS)	25g / 500g [S0588]
***N,N,N',N'*-Tetramethylethylenediamine (= TEMED)**	5g / 25g [T2515]
Tris(hydroxymethyl)aminomethane (= Tris-Base)	25g / 500g [T2516]

Protein Staining Reagent

Coomassie Brilliant Blue G-250 (Ready-to-use solution) [for Electrophoresis]
500mL [C3488]

Application

1. After electrophoresis, wash the gel with deionized water for 5 minutes three times.
2. Remove the water used for washing, add C3488 till the gel is soaked, and let the gel stain for 1 hour while shaking gently at room temperature.
3. Remove the staining solution, destain the gel with deionized water for 1 hour and check it.
4. If the background is high, destain the gel with deionized water overnight at room temperature.

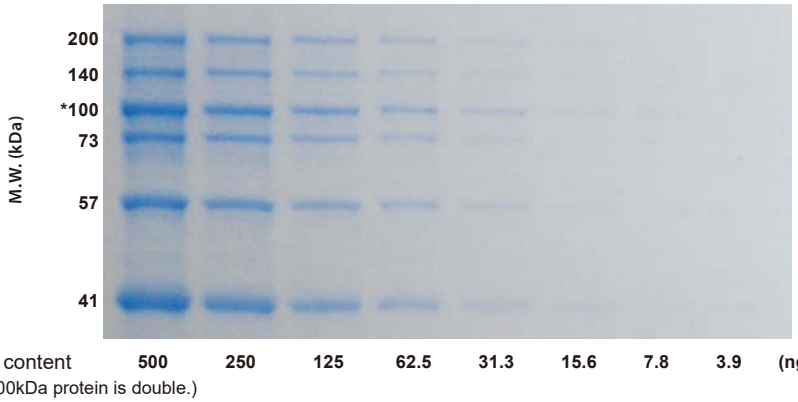


Figure. Proteins stained by the above method (destained overnight)

Reagent for Gel Staining

Gel Negative Stain kit 1kit [G0615]

Reagents for Protein Staining and Others

Acid Black 1 (= Amido Black 10B)	5g [A2097]
Acid Red 112 (= Ponceau S)	1g / 5g [A2256]
Coomassie Brilliant Blue G-250	5g [B3193]
Coomassie Brilliant Blue R-250	5g [B3194]
Fast Green FCF	5g [F0718]
Sodium Deoxycholate	25g [D1820]
6-Aminohexanoic Acid	5g / 25g [A2255]

Nucleic Acid Detecting Reagents

Nucleic Acid Staining Reagent

Ethidium Bromide (0.5mg/mL in Water) (in Dropper Bottle) [for Electrophoresis] 10mL [E1363]

It can be used to stain nucleic acids after agarose gel electrophoresis. Since the nucleic acids are stained blue, no transilluminator or other detection device is required. Unlike ethidium bromide, it is non-mutagenic and therefore safe and easy to handle.

Each drop contains 20 µg of Ethidium Bromide, so you can easily adjust the solution as final concentration. Convenient and safe to use because of dropper bottle.

Application

After electrophoresis, dilute E1363 (1 drop / 40 mL) to 0.5 µg/mL with water or running buffer and incubate the gel for 15 minutes. If you have to decrease background fluorescence, wash the gel in water for 15 minutes. In use of electrophoresis buffer solution, Ethidium Bromide incorporated into nucleic acid and can visualize band immediately by using UV transilluminator.

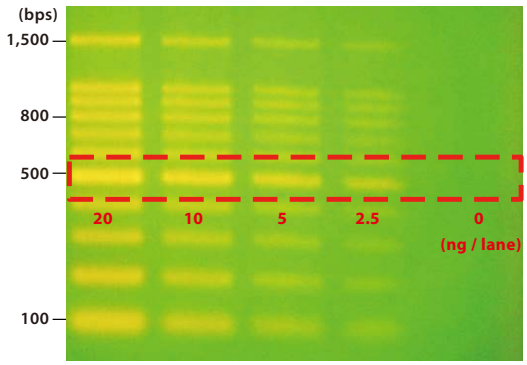


Figure. DNA Ladder Marker stained by the above method (destained 15 min)

10X Nucleic Acid Stain Blue 100mL [N1209]

Nucleic Acid Sample Preparation Reagents for Electrophoresis

6X Loading Buffer Bromophenol Blue	(1 mL×3) 1set 4,000 円 [L0393]
6X Loading Buffer Double BX	(1 mL×3) 1set 4,000 円 [L0440]

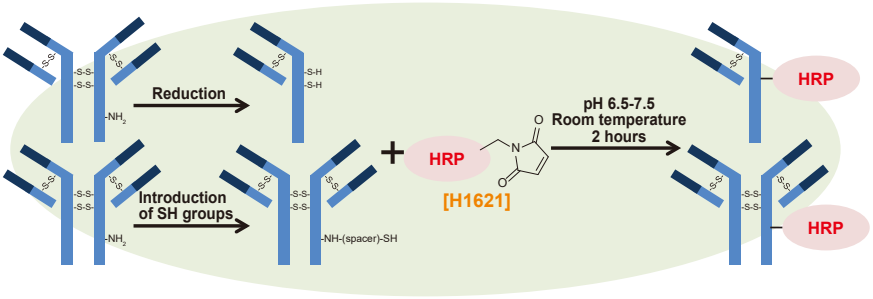
Protein-maleimide Conjugates for Thiol-maleimide Crosslinking

Bovine Serum Albumin Maleimide Conjugate (1mg×3)	1set [B5944]
Horseradish Peroxidase Maleimide Conjugate (0.5mg×3)	1set [H1621]
Streptavidin Maleimide Conjugate (0.5mg×1)	1vial [T3531]

Each product containing a thiol-reactive maleimide group can be used for the conjugation to proteins and peptides containing free thiols. Each protein conjugate is packaged for single use purposes and thus does not require weighing prior to use.

Application : HRP-labelling of an antibody with H1621

In case of antibodies without free thiol (SH, sulfhydryl) groups, disulfide moieties in proteins can be reduced by a reductant such as DTT [D3647] or 2-MEA [A0296] to reveal free thiols.
Furthermore, thiol group can be introduced to primary amines by adding SATA [S0431], SATP [S0859] or 2-Iminothiolane.

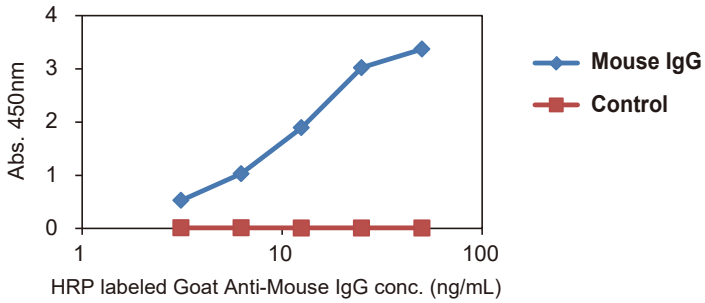


Example protocol for antibody conjugation starts from a reduction of native disulfide bonds in the Goat Anti-Mouse IgG, followed by labeling with the HRP using H1621. For more information, see the product detail page of H1621 on TCI website.

Protocol

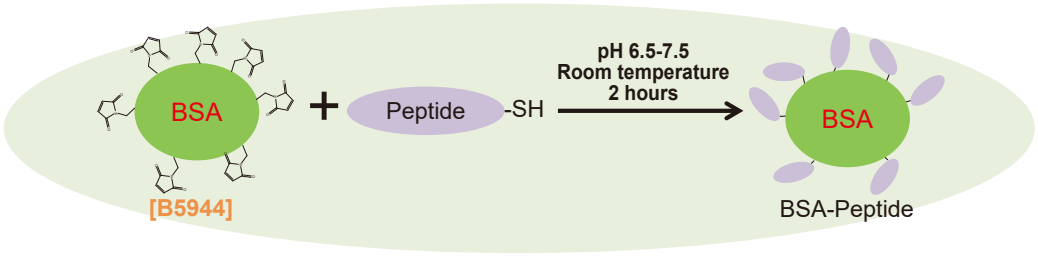
1. Add DTT to a final concentration equal to 3 mole equivalents per mole equivalent of antibody present.
2. Incubate for 90 minutes at 37 °C.
3. Purify the reduced IgG by gel filtration or ultrafiltration, dialysis.
4. Add equal amount of H1621 (by weight) to a purified antibody and Incubate for 2 hours at room temperature (25 °C).

Activity of HRP labeled antibody



Goat Anti-Mouse IgG labeled with the HRP using H1621 was tested by ELISA for detection of a Mouse IgG coated on a plate. Mouse IgG could be detected sufficiently even if the labeled antibody was diluted to 5 ng/mL or more.

Application : Preparation of BSA-Peptide using B5944

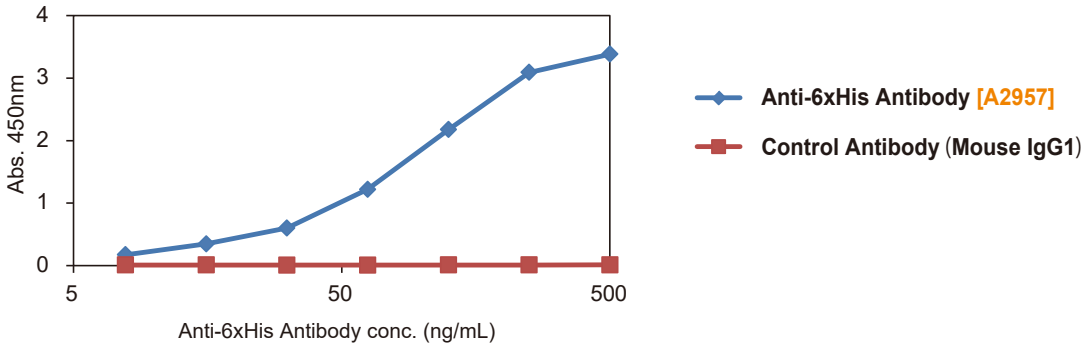


BSA is conjugated to haptens and typically used as an antigen carrier for anti-hapten antibody.
Here we show how to conjugate 6xHis-Cys peptide to **B5944**.
For more information, see the product detail page of **B5944** on TCI website.

Protocol

1. Dissolve the 6xHis-Cys peptide in 0.1 M sodium phosphate, 0.15 M NaCl, 0.1 M EDTA at pH 7.2.
2. Reconstitute the **B5944** with 100 µL of water.
3. Add 1mg of 6xHis-Cys peptide to 1 mg of **B5944** and Incubate for 2 hours at room temperature (25 °C).

ELISA using the prepared BSA-6His as an antigen



Anti-6xHis Antibody **[A2957]** was analysed by ELISA using a 0.1 µg / well of BSA-6His coated plate.
Goat Anti-Mouse IgG HRP Conjugate **[G0407]** was used as the secondary antibody.

Related Products

Reducing agents for protein disulfide

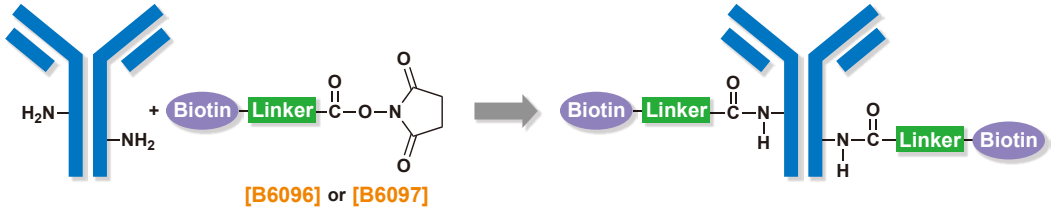
DTT (= DL-Dithiothreitol)	1g / 5g **[D3647]**
2-MEA (= 2-Aminoethanethiol Hydrochloride)	25g / 100g / 500g **[A0296]**
2-Mercaptoethanol	5g / 25g **[M1948]**
Tris(2-carboxyethyl)phosphine Hydrochloride	1g / 5g / 25g **[T1656]**

Reagents for introduction of thiol group

SATA (= *N*-Succinimidyl *S*-Acetylthioglycolate)	1g / 5g **[S0431]**
SATP (= *N*-Succinimidyl 3-(Acetylthio)propionate)	100mg **[S0859]**

Pre-Weighed Biotinylation Reagents

Biotin-LC-LC-NHS (2mg×5) 1set [B6096]
Biotin-PEG$_2$-NHS (2mg×5) 1set [B6097]



[B6096] or [B6097]

B6096 and B6097 contain both a linker and an *N*-hydroxysuccinimidyl ester moiety, and easily react with amino group (-NH$_2$) of proteins. Target samples can be biotinylated without weighing of the products during the preparation. B6096 and B6097 include 5 reagent vials, each containing 2 mg of respective reagent. The pre-aliquoted packaging prevents decline of the reagent reactivity over time by eliminating the need for repetitive opening of the vial.

Applications

Preparation :

Use of a 10 mM biotinylation solution is recommended. In order to efficiently biotinylate a sample, biotinylation solution should be used at a 15-fold molar excess over the amount of amine-containing protein. Make sure to calculate the 10 mM biotinylation solution amount (see example below).

Calculate : A µL of 10 mM biotinylation solution for biotinylation of 2 mg IgG (150,000 M.W.)

2 [mg IgG] x 10^{-3} [g/mg] x 1/150,000 [mol/g] x 15 [fold]
= A [µL of 10 mM biotinylation solution] x 10^{-6} [L/µL] x 10 [mmol/L] x 10^{-3} [mol/mmol]

A = 20 [µL of 10 mM biotinylation solution]

Direction for Use :

1. Bring each product to room temperature.
2. Dissolve 2 mg of Biotin-LC-LC-NHS [B6096] in 350 µL of DMSO or DMF or 2 mg of Biotin-PEG$_2$-NHS [B6097] in 400 µL of PBS to prepare a 10 mM biotinylation solution.
3. Dissolve the sample (1-10 mg/mL) in an appropriate buffer such as PBS. Do not use buffers including amines (such as Tris).
4. Add A µL of 10 mM biotinylation solution to the sample solution and incubate the mixed solution for 30 min at room temperature.
5. Remove unreacted and hydrolyzed reagent using desalting column or dialysis methods.

Related Products

Biotin-LC-LC-NHS	25mg / 100mg [S0956]
Biotin-PEG$_2$-NHS	25mg / 100mg [S0955]
Biotin-PEG$_2$-Maleimide	50mg [B3174]
Streptavidin from *Streptomyces avidinii*	1mg/vial [S0951]
Streptavidin HRP Conjugate	0.1mg/vial [S0972]
Streptavidin FITC Conjugate	0.1mg/vial [S0966]
Streptavidin Maleimide Conjugate	0.5mg/vial [T3531]
HABA	5g / 25g [H0586]
Sulfo-SMCC Sodium Salt	20mg / 100mg [S0883]
Horseradish Peroxidase Maleimide Conjugate (0.5mg×3)	1set [H1621]
BSA Maleimide Conjugate (1mg×3)	1set [B5944]

Extraction Buffer for Mammalian Cells

RIPA Buffer (Ready-to-use) [for Protein extraction] 100mL [R0246]

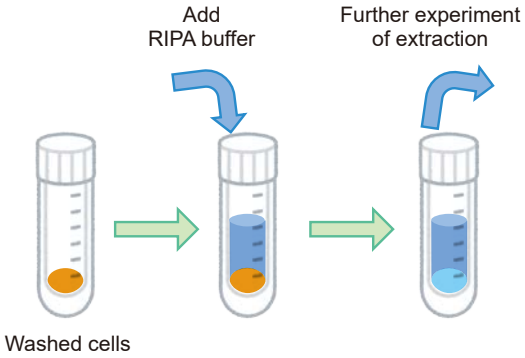
This product is supplied as a ready-to-use solution for the lysis of the cultured mammalian cells. Proteins can be extracted by adding this buffer [R0246] to the cells and the extract can be used directly for further analysis such as western blotting. This product does not include protease inhibitors. Please add a protease inhibitor cocktail, if necessary.

Application

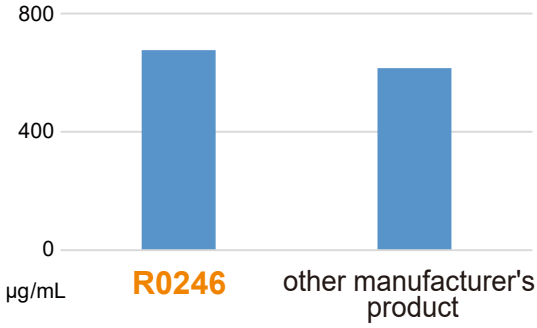
Add the following protease inhibitors to RIPA buffer [R0246].

Leupeptin	10 µg/mL
Pepstatin A	1 µg/mL
Aprotinin	3 µg/mL
AEBSF	1 mM

1. Wash the cultured mouse myeloma-derived cell sp2/0 twice with PBS.
2. Remove PBS and add 200 µL of either cold RIPA buffer [R0246] containing protease inhibitors or the other manufacturer's RIPA buffer containing the same protease inhibitors to 1.0 x 10^6 cells.
3. Incubate the cells for 15 minutes on ice.
4. Centrifuge the cells at 10000 x g for 10 minutes at 4 °C
5. Measure the protein concentration of the supernatants.
6. Analyze the supernatants using western blotting.



Extracted Protein Concentration



The extracts were transferred to a PVDF membrane after electrophoresis.
Anti-β actin antibody was used for detection.
Equal or better detection was observed than that of the other manufacturer's product.

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TCI AMERICA
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Fax : 888-520-1075 / 503-283-1987
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E-mail : Sales-EU@TCIchemicals.com

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Fax : +49 (0)6196 64053-01
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E-mail : Sales-UK@TCIchemicals.com

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Tel : +81 (0)3-5640-8878
E-mail : globalbusiness@TCIchemicals.com