Reagents for Antibody Detection

Unlabeled Secondary Antibodies

Anti-Goat IgG (whole molecule)

G 4018 **antibody produced in rabbit** 1 mL
–20°C **Affinity isolated antibody, Buffered**
◆◆ **aqueous solution**
DRY ICE Binds all goat Igs.
Solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide
Application(s)
Quantitative precipitin assay. 2.0

Anti-Mouse IgG (Fc specific)

M 2650 **antibody produced in goat** 1 mL
–20°C **Affinity isolated antibody, Buffered**
◆◆ **aqueous solution**
DRY ICE Binds mouse IgG; does not bind other mouse Igs.
Solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide
Adsorbed to reduce background staining with human samples.
Application(s)
Quantitative precipitin assay. . . . . .2.0 mg/mL

Anti-Rabbit IgG (whole molecule)

R 2004 **antibody produced in goat** 1 mg
2–8°C **Affinity isolated antibody,** 5 × 1 mg
Lyophilized powder
Binds all rabbit Igs.
Lyophilized from 0.01 M sodium phosphate, 0.015 M sodium chloride, pH 7.2

Antibody-Alkaline Phosphatase Conjugates

Anti-Goat IgG (whole molecule)–Alkaline Phosphatase

A 4187 **antibody produced in rabbit** 0.25 mL
2–8°C **Affinity isolated antibody, Buffered** 0.5 mL
◆◆ **aqueous glycerol solution** 1 mL
WET ICE Binds all goat Igs
Solution in 0.05 M Tris buffer, pH 8.0, containing 1 mM $MgCl_2$, 10 mM glycine, 1% bovine serum albumin, 50% glycerol and 15 mM sodium azide
Application(s)
Immunoblotting. 1:30,000
Immunohistochemistry (formalin-fixed, paraffin-embedded sections). 1:50
Direct ELISA. 1:30,000

Anti-Mouse IgG (whole molecule)-Alkaline Phosphatase

A 3562 **antibody produced in goat** 0.25 mL
2–8°C **Affinity isolated antibody, Buffered** 0.5 mL
◆◆ **aqueous glycerol solution** 1 mL
WET ICE Binds all mouse Igs
Solution in 0.05 M Tris buffer, pH 8.0, containing 1 mM $MgCl_2$, 10 mM glycine, 1% bovine serum albumin, 50% glycerol and 15 mM sodium azide
Application(s)
Immunoblotting. 1:30,000
Immunohistochemistry (formalin-fixed, paraffin-embedded sections). 1:50
Direct ELISA. 1:30,000

Anti-Rabbit IgG (whole molecule)–Alkaline Phosphatase

A 3812 **antibody produced in goat** 0.25 mL
2–8°C **Affinity isolated antibody, Buffered** 0.5 mL
◆◆ **aqueous glycerol solution** 1 mL
WET ICE Binds all rabbit Igs
Solution in 0.05 M Tris buffer, pH 8.0, containing 1 mM $MgCl_2$, 10 mM glycine, 1% bovine serum albumin, 50% glycerol and 15 mM sodium azide
Adsorbed to reduce background staining with human samples.
Application(s)
Immunoblotting. 1:30,000
Immunohistochemistry (formalin-fixed, paraffin-embedded sections). 1:50
Direct ELISA. 1:30,000

Antibody-Peroxidase Conjugates

Anti-Goat IgG (whole molecule)–Peroxidase

A 5420 **antibody produced in rabbit** 1 mL
–20°C **Affinity isolated antibody, Buffered**
◆◆ **aqueous solution**
DRY ICE Binds all goat Igs
Solution in 0.01 M phosphate buffered saline, pH 7.4, containing 0.01% thimerosal
Application(s)
Indirect immunoblotting (chemiluminescent). 1:80,000
Immunohistochemistry (formalin-fixed, paraffin-embedded sections). 1:200
Direct ELISA. 1:40,000
Dot blot. 1:8,000

Anti-Mouse IgG (Fab specific)–Peroxidase

A 9917 **antibody produced in goat** 1 mL
–20°C **Affinity isolated antibody, Buffered**
◆◆ **aqueous solution**
DRY ICE Antibody adsorbed with human IgG.
Solution in 0.01 M phosphate buffered saline pH 7.4, containing 0.01% thimerosal
Prepared by the two-step glutaraldehyde method described by Avrameas, S., et al., Scand. J. Immunol., **8**, Suppl. 7, 7 (1978).
Application(s)
Indirect immunoblotting (chemiluminescent). 1:80,000
Immunohistochemistry (formalin-fixed, paraffin-embedded sections). 1:150
Direct ELISA. 1:60,000
Dot blot. 1:8,000

Anti-Mouse IgG (Fc specific)–Peroxidase

A 0168 **antibody produced in goat** 1 mL
–20°C **Affinity isolated antibody, Buffered**
◆◆ **aqueous solution**
DRY ICE Binds mouse IgG; does not bind other mouse Igs.
Solution in 0.01 M phosphate buffered saline pH 7.4, containing 0.01% thimerosal
Adsorbed to reduce background staining with human samples.
Application(s)
Indirect immunoblotting (chemiluminescent). 1:6,000-1:80,000
Immunohistochemistry (formalin-fixed, paraffin-embedded sections). 1:100
Direct ELISA. 1:50,000
Dot blot. 1:4,000

Reagents for Antibody Detection

Antibody-Peroxidase Conjugates

Anti-Rabbit IgG (whole molecule)–Peroxidase

A 0545 **antibody produced in goat** 1 mL
–20°C **Affinity isolated antibody, Buffered aqueous solution**
DRY ICE Binds all rabbit Igs
Solution in 0.01 M phosphate buffered saline, pH 7.4, containing 0.01% thimerosal
Adsorbed to reduce background staining with human samples.
Application(s)
Indirect immunoblotting (chemiluminescent). 1:160,000
Immunohistochemistry (formalin-fixed, paraffin-embedded sections). 1:300
Direct ELISA. 1:40,000
Dot blot. 1:16,000

Antibody-Fluorochrome Conjugates

Anti-Goat IgG (whole molecule)–Cy3

C 2821 **antibody produced in rabbit** 1 mL
2–8°C **Affinity isolated antibody, Buffered aqueous solution**
WET ICE Binds all goat Igs.
Solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide
Application(s)
Immunohistochemistry (formalin-fixed, paraffin-embedded sections). 1:50
Cy is distributed under license from Amersham Biosciences Limited.

Anti-Goat IgG (whole molecule)–FITC

F 7367 **antibody produced in rabbit** 0.5 mL
–20°C **Affinity isolated antibody, Buffered aqueous solution** 1 mL
2 mL
Binds all goat Igs.
Solution in 0.01 M phosphate buffered saline, pH 7.4, containing 0.01% thimerosal
Application(s)
Immunohistochemistry (formalin-fixed, paraffin-embedded sections). 1:400
Indirect immunofluorescence. 1:400

Anti-Mouse IgG (whole molecule)–FITC

F 0257 **antibody produced in goat** 0.5 mL
2–8°C **Affinity isolated antibody, Buffered aqueous solution** 1 mL
2 mL
Binds all mouse Igs. 5 × 2 mL
Solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide
Application(s)
Direct immunofluorescence. 1:32

Anti-Mouse IgG (whole molecule) F(ab′)$_2$ fragment–Cy3

C 2181 **antibody produced in sheep** 1 mL
2–8°C **Affinity isolated antibody, Buffered aqueous solution**
WET ICE Binds all mouse Igs
Useful when trying to avoid background staining due to the presence of Fc receptors.
Solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide
Adsorbed to reduce background staining with human samples.
Application(s)
Immunohistochemistry (formalin-fixed, paraffin-embedded sections). 1:50
Cy is distributed under license from Amersham Biosciences Limited.

Anti-Rabbit IgG (whole molecule)–FITC

F 0382 **antibody produced in goat** 0.5 mL
2–8°C **Affinity isolated antibody, Buffered aqueous solution** 1 mL
2 mL
Binds all rabbit Igs.
Solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide
Application(s)
Indirect immunofluorescence. 1:40

Anti-Sheep IgG (whole molecule)–FITC

F 7634 **antibody produced in donkey** 0.5 mL
2–8°C **Affinity isolated antibody, Buffered aqueous solution** 1 mL
2 mL
Binds all sheep Igs
Solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide
Application(s)
Indirect immunofluorescence. 1:40

Avidin / Biotin Reagents

ExtrAvidin®

E 2511 **Essentially salt-free, lyophilized powder** 1 mg
2–8°C A modified form of affinity purified egg white 5 mg
WET ICE avidin.
Binding Activity: at least 10 µg biotin per mg

ExtrAvidin®–Alkaline Phosphatase

E 2636 **Buffered aqueous solution** 0.2 mL
2–8°C Solution in 0.05 M Tris-HCl buffer, 0.5 mL
WET ICE pH 8.0, containing 1 mM $MgCl_2$, 1% 5 × 0.5 mL
bovine serum albumin and 15 mM sodium azide
Affinity purified protein
Application(s)
Indirect immunoblotting (chemiluminescent). 1:300,000
Immunohistochemistry (formalin-fixed, paraffin-embedded sections). 1:100
Indirect ELISA. 1:70,000
Dot blot. 1:300,000

ExtrAvidin®–Cy3

E 4142 **Buffered aqueous solution** 1 mL
2–8°C Solution in 0.01 M phosphate buffered saline,
WET ICE containing 1% bovine serum albumin and 15 mM sodium azide
Affinity purified protein
Extent of labeling. . . . . 3-9 mol Cy3 per mol protein
Application(s)
Immunohistochemistry (formalin-fixed, paraffin-embedded sections). 1:100
Cy is distributed under license from Amersham Biosciences Limited.

ExtrAvidin®–FITC

E 2761 **Buffered aqueous solution** 0.2 mL
–20°C Solution in 0.01 M phosphate buffered 1 mL
DRY ICE saline, containing 10% (v/v) 0.5 M carbonate buffer, pH 9.5, and 15 mM sodium azide
Affinity purified protein
Extent of labeling. . . . . 3-5 mol fluorochrome per mol protein
Application(s)
Indirect immunofluorescence. 1:200

Reagents for Antibody Detection

Avidin / Biotin Reagents

ExtrAvidin®–Peroxidase

E 2886 2–8°C ◆◆ WET ICE — **Buffered aqueous solution** — 0.2 mL

Solution in 0.01 M phosphate buffered saline, pH 7.4, containing 0.01% thimerosal — 1 mL; 5 × 1 mL

Affinity purified protein

Application(s)

Immunohistochemistry (formalin-fixed, paraffin-embedded sections). 1:50
Direct ELISA. 1:1,000
Dot blot. 1:2,000

Streptavidin Reagents and Kit for detection See: Streptavidin Reagents *Page* 56

Antibody Purification

Protein A Antibody Purification Kit

PURE-1A 2–8°C ◆◆ WET ICE — 1 kit

Using our cartridge system, antibodies elute as highly purified proteins at physiological pH. Protein A is a powerful tool for isolation of antibodies from mammalian hosts. Protein A exhibits a high degree of specificity for IgG and ensures an antibody preparation virtually free of IgA, IgM and non-immunoglobulin serum proteins such as albumin. PURE-1A offers Protein A technology in a prepackaged, easy to use kit form. With this kit, milligram quantities of IgG can be purified from serum, ascites, or cell culture supernatants.

Purification of IgG from other species may be possible, however, the researcher will have to determine the suitability of the kit for their application.

Features and Benefits

- High capacity - purify up to 8 mg of mouse IgG or 25 mg of human IgG per column run
- Specific - will only bind IgG
- Easy to use - antibody is eluted and desalted in a single step, ready to use
- Gentle - avoids prolonged exposure of the antibody to low pH

Capacity/Run (by Species)

Human IgG 20-25 mg
Mouse IgG1 8-12 mg
Mouse IgG2a 10-15 mg
Mouse IgG2b 6-10 mg
Mouse IgG3 15-20 mg
Rabbit IgG 8-12 mg
Goat IgG 2-4 mg
Bovine IgG 8-12 mg
1 kit sufficient for 10 purifications

Components:

Binding Buffer, 225 mL
Desalting Cartridge, 5 mL
Directions for Use,
Elution Buffer, 75 mL
HEPES Buffer, 225 mL
5 ml Syringe, 1
10 ml Syringe, 1
Protein A Cartridge, 1 mL
Regeneration Buffer, 75 mL

Protein G Immunoprecipitation Kit

IP-50 2–8°C ◆◆ — 1 kit

The kit is designed to allow maximal recovery of immunoprecipitates. It provides all the necessary reagents to perform immunoprecipitation from cell extracts of any protein to which a suitable antibody is available. Based on Protein-G, the kit binds to most commonly used antibodies. In addition, spin columns are provided to enable quick washes without the loss of protein-G resin and thus protein yield is maximized.

Features and Benefits

- Minimal loss of antigen-antibody bound beads during washing.
- Minimal or no non-specific signals by increasing the stringency of the washing step.

1 kit sufficient for 50 reactions immunoprecipitation

Components:

10% Sodium dodecyl sulfate solution, 1 mL
10× IP Buffer, 40 mL
Microcentrifuge tubes (2 ml), 50 each
Protein G Agarose, 2 mL
5 M Sodium chloride solution, 15 mL
Spin columns and caps, 50 each

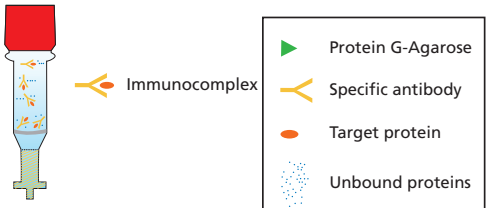
R: 10-22-37/38-41 S: 16-26-36/39

Reagents for Antibody Detection

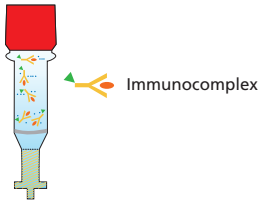
Antibody Purification

IP-50 Protein G Immunoprecipitation Procedure

Step 1. Formation of antigen-antibody complex: Mix cell lysate with antibody

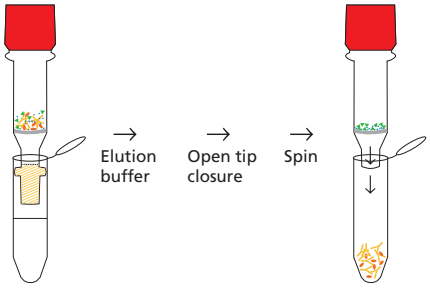


Step 2. Immunoprecipitation: Add Protein G-Agarose,



Step 3. Removal of non-specific binding: Snap off column tip, place in microfuge tube. Spin and wash extensively.

Step 4. Elution of the immunoprecipitated proteins (Inverted broken tip serves as column closure)



Protein A–Agarose

P 7786 2–8°C
from *Staphylococcus aureus* 1 mL
Aqueous suspension 5 mL
Suspension in 0.9% NaCl containing 0.02% thimerosal 10 mL
Matrix. . . . . Cross-linked 4% beaded agarose
Matrix activation. . . . . p-nitrophenyl chloroformate
Matrix attachment. . . . . amino
Matrix spacer. . . . . 1 atom
binding capacity (human IgG). . . . . approx.10 mg/mL

Protein A–Sepharose

P 3391 2–8°C
from *Staphylococcus aureus* 250 mg
Lyophilized powder 1 g
Lyophilized powder stabilized with lactose and dextran 1.5 g
Swelling: 1 g swells to approx. 4 ml 5 g
Extent of labeling. . . . . approx.2 mg per mL
Matrix. . . . . Sepharose CL-4B
Matrix activation. . . . . cyanogen bromide
Matrix attachment. . . . . amino
Matrix spacer. . . . . 1 atom
binding capacity (human IgG). . . . . approx.20 mg/mL

Protein G Agarose, Fast Flow

P 4691 2–8°C
from *Streptococcus* sp. 1 mL
Saline suspension 5 mL
Suspension in 0.5 M NaCl containing 20% ethanol as a preservative
Prepared with a genetically engineered Protein G which retains its high affinity for IgG and lacks albumin and Fab binding sites and membrane binding regions.
Extent of labeling. . . . . approx.4 mg per mL
Matrix activation. . . . . cyanogen bromide
Matrix attachment. . . . . amino
Matrix spacer. . . . . 1 atom
binding capacity (human IgG (I 4506)). . approx.30 mg/mL (in 20 mM sodium phosphate, pH 7.0)
Matrix. . . . . Highly cross-linked 4% beaded agarose, fast flow
References
1. Åkerström, B., and Björck, L., *J. Biol. Chem.* **261**, 10240-10247 (1986)
2. Shimazaki, Y., et al., J., *J. Biochem. Biophys. Meth.* **37**, 1-4 (1998)

Protein G Sepharose, Fast Flow

P 3296 2–8°C ◆
from *Streptococcus* sp. 1 mL
recombinant, expressed in *Escherichia coli*, Aqueous ethanol suspension 5 mL
Suspension in 20% ethanol
Prepared with recombinant streptococcal protein G from which the albumin-binding region has been genetically deleted
Extent of labeling. . . . . approx.2 mg per mL
Matrix. . . . . Sepharose 4B Fast Flow
Matrix activation. . . . . cyanogen bromide
Matrix attachment. . . . . amino
Matrix spacer. . . . . 1 atom
binding capacity (human IgG). . . . . >20 mg/mL
R: 10-36/37/38 S: 16-26-36

Protein L–Agarose

P 3351 2–8°C ◆
from *Peptostreptococcus magnus* 5 mL
recombinant, expressed in *Escherichia coli*
Protein L from *Peptostreptococcus magnus* binds immunoglobulins (Ig) primarily through kappa light chain interactions without interfering with the antigen binding site. Recombinant Protein L contains four Ig-binding domains.
Prepared with recombinant *Peptostreptococcus magnus* Protein L.
Extent of labeling. . . . . 1-2 mg per mL
Matrix. . . . . Cross-linked 4% beaded agarose
Matrix activation. . . . . cyanogen bromide
Matrix attachment. . . . . amino
binding capacity . . . . . 3-10 mg/mL
References
1. Bjorck, L., Protein L. A novel bacterial cell wall protein with affinity for Ig L chains *J. Immunol.* **140**, 1194-1197 (1988)
2. Kastern, W. et al., et al., Structure of peptostreptococcal protein L and identification of a repeated immunoglobulin light chain-binding domain *J. Biol. Chem.* **267**, 12820-12825 (1992)
R: 10-36/37/38 S: 26-36

Reagents for Antibody Detection

Antibody Modification

BiotinTag™ Micro Biotinylation Kit

B-TAG 2–8°C — 1 kit

For custom biotinylation of proteins, Sigma offers kits for conjugation on two different scales. The biotin-avidin system has become popular when high sensitivity and specificity are desired. Biotin can be conjugated to antibodies, lectins, enzymes, and other proteins. The protocols in the kits have been optimized for antibodies. Avidin binds to biotin with a high affinity ($K_a = 10^{15}$ M) and specificity. When conjugated to enzymes or fluorchromes avidin provides a means of identifying biotinylated compounds via enzymatic conversion of substrate to form a visible product or detection of fluorescence by spectrophotometry or flow cytometry.

Biotin has been modified with aminocaproate, then activated via an ester linkage with sulfo-N-hydroxysuccinimide (BAC-Sulfo-NHS). Aminocaproate provides a six-carbon spacer that reduces steric hindrance on the biotin and improves accessibility to the binding site on avidin. Sulfonation of the hydroxysuccinimide increases the polarity of the reagent, allowing it to dissolve easily in aqueous buffer. The ester provides a carbonyl carbon adjacent to a labile ester linkage as a target for primary amine side chains of accessible lysine residues, joining the biotinamidocaproate to the protein via an amide bond (see reaction).

Features and Benefits

- Complete protocols for labeling and assay
- BAC-Sulfo-NHS is completely water soluble
- no DMF or DMSO needed
- Biotinylation occurs near neutral pH and physiological ionic strength, avoiding harsh conditions that could damage sensitive proteins
- Fast separation of conjugate from reactants using gel filtration
- 2 - 5 molar ratio biotin to protein in conjugate (for antibodies)
- Two scales to choose from:
- 1 mg protein per reaction (B-TAG)
- 10 mg protein per reaction (BK-101)
- Sufficient reagents for at least 5 labelings

Procedure:

Conjugation is performed in four easy steps:

1. Reconstitute BAC-Sulfo-NHS with Phosphate Buffer (PB).
2. Add BAC-Sulfo-NHS to protein and allow to react for 30 min at room temperature.
3. Separate the conjugate from the reactants on gel filtration.
4. Assay the conjugate for biotin incorporation by the avidin-HABA assay (BK-101 only). Conjugate is ready to use.

Components:

0.01 M PBS, pH 7.4, powder, 1 × 1 L
0.1 M Phosphate Buffer, pH 7.2, powder, 1 × 5 mL
BAC-Sulfo-NHS, 1 × 5 mg
ExtrAvidin® Peroxidase, 1 × 0.2 mL
G-50 Micro-spin Column, 5 each

References

1. Bartoli, M., et al., Interaction of calmodulin with striatin, a WD-repeat protein present in neuronal dendritic spines *J. Biol. Chem.* **273**, 22248-22253 (1999)
2. Green, N.M., A spectrophotometric assay for avidin and biotin based on binding of dyes by avidin *Biochem. J.* **94**, 23c-24c (1965)
3. Duk, M. et al., The biotin/avidin-mediated microtiter plate lectin assay with the use of chemically modified glycoprotein ligand. *Anal. Biochem.* **221**, 266-272 (1994)
4. Ignatowski, T.A., and Bidlack, J.M., Differential kappa-opioid receptor expression on mouse lymphocytes at varying stages of maturation and on mouse macrophages after selective elicitation *J. Pharmacol. Exp. Ther.* **290**, 863-870 (1999)
5. Rao S.V., et al., Controlled layer-by-layer immobilization of horseradish peroxidase *Biotechnol. Bioeng.* **65**, 389-396 (1999)

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

FluoroTag™ FITC Conjugation Kit

FITC-1 2–8°C — 1 kit

Sigma offers a convenient kit for preparing FITC-labeled antibodies. Fluorescein isothiocyanate (FITC), Isomer 1, is a widely used fluorophore, popular because of its high quantum efficiency and stability when conjugated. FITC is yellow-orange in color with an absorption maximum at 495 nm. Upon excitation it emits a yellow-green color with an emission maximum at 525 nm. Conjugation occurs through free amino groups of proteins or peptides, forming a stable thiourea bond (see reaction). FITC conjugates of antibodies, lectins, hormones, and growth factors have been used in a variety of immunohistochemical and flow cytometry applications. The protocols have been optimized for antibodies, but may be adapted to other proteins by the end user.

Features and Benefits

- Suitable for both small (1 mg) and large (5 mg) scale conjugations
- Completely aqueous procedure - no DMF needed
- Fast gel filtration separation of conjugate from excess FITC
- Complete protocols for conjugation and F/P ratio determination
- Sufficient reagents for at least 5 conjugations of 5 mg protein each and for optimization of F/P ratio before scale-up
- References for applications and protocols

Procedure

1. Dissolve protein and FITC in carbonate-bicarbonate buffer.
2. Slowly add FITC to protein with stirring. Cover with foil and stir 2 hours at room temperature.
3. Separate conjugate from free FITC on G-25 column. Collect fractions.
4. Pool fractions containing conjugate.
5. Determine F/P ratio of conjugate spectrophotometrically.
6. Stabilize with 1% bovine serum albumin and 0.1% sodium azide and store at 0-5 °C.

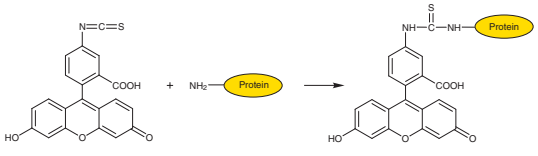
Components:

0.1 M carbonate-bicarbonate buffer, pH 9.0, 5 capsules
FITC, Isomer I, 5×2 mg
PBS, pH 7.4, 5 packages
Sephadex G-25 column, 3.5 ml, 1
Sephadex G-25 column, 9.1 ml, 1

References

1. Sender, S., et al., Localization of carbonic anhydrase IV in rat and human heart muscle. *J. Histochem. Cytochem.* **46**, 855-861 (1998)
2. Mullins, R.F., et al., Characterization of drusen-associated glycoconjugates *Ophthalmology* **104**, 288-294 (1997)
3. Kobayashi, Y., et al., Effects of a GnRH analogue on human smooth muscle cells cultured from normal myometrial and from uterine leiomyomal tissues. *Mol. Hum. Reprod.* **3**, 91-99 (1997)
4. Blackmore P.F., Extragenomic actions of progesterone in human sperm and progesterone metabolites in human platelets *Steroids* **64**, 149-156 (1999)
5. Hidaka, H., et al., The identification of specific high density lipoprotein3 binding sites on human blood monocytes using fluorescence-labeled ligand. *J. Lipid Res.* **40**, 1131-1139 (1999)
6. Lloyd-Evans, P., et al., Use of a phycoerythrin-conjugated anti-glycophorin A monoclonal antibody as a double label to improve the accuracy of FMH quantification by flow cytometry. *Transfus. Med.* **9**, 155-160 (1999)

R: 20/21/22-42/43 S: 26-36



Conjugation Reaction.

Reagents for Antibody Detection

Antibody Modification

Fluorescence Marker Kit 550

92813 2-8°C ◆ NEW — ***BioChemika*** — 1 kit

For fluorescent labelling of proteins, peptides, and amino-modified nucleotides. Comprises phosphate buffer solution, bicarbonate buffer solution, and Fluorescent Orange 548 reactive.
Sufficient for 5 reactions labelling 1.5 mg of protein each.
This kits provides the required reagents for labelling proteins with Fluorescent orange 548. This label shows similar properties as Cy® 3. This means among others bright fluorescence, spectral match with corresponding laser line, good water solubility. Together with Fluorescent red 646 it is ideally suited for energy transfer studies, hybridization assays and flow cytometry.
® Registered Trademark of Amersham Biosciences Limited

Fluorescence Marker Kit 650

92821 2-8°C ◆ NEW — ***BioChemika*** — 1 kit

For fluorescent labelling of proteins, peptides, and amino-modified nucleotides. Comprises phosphate buffer solution, bicarbonate buffer solution, and Fluorescent Orange 646 reactive.
Sufficient for 5 reactions labelling 1.5 mg of protein each.
This kits provides the required reagents for labelling proteins with Fluorescent red 646. This label shows similar properties as Cy® 5. This means among others bright fluorescence, spectral match with corresponding laser line, good water solubility. Together with Fluorescent orange 548 it is ideally suited for energy transfer studies, hybridization assays and flow cytometry.
® Registered Trademark of Amersham Biosciences Limited

Fluorescein isothiocyanate isomer I

F 7250 2-8°C — (Fluorescein 5-isothiocyanate; FITC)

CAS No. 3326-32-7
$C_{21}H_{11}NO_5S$ FW 389.4
suitable for protein labeling, minimum90% (HPLC), powder
Fluorescence. . . . . λ_{ex} 492 nm; λ_{emm} 518 nm (green)
Color. . . . . green

50 mg
100 mg
250 mg
500 mg
1 g
5 g

References
E. Harlow and D. Lane, ed., *Antibodies: A Laboratory Manual* , Cold Spring Harbor, NY (1988), 353-355
R: 42 S: 22-24/25

StarBright® Green Isothiocyanate Protein Labeling Kit

MT3000 2-8°C ◆ WET ICE NEW — 1 kit

The Isothiocyanate-StarBright® Green Conjugation Kit is suitable for the conjugation of proteins such as polyclonal and monoclonal antibodies for use in immunohistochemical or immunofluorescent techniques. In addition, the ITCN conjugation kit may also be used with peptide hormones, cytokines, growth factors and other proteins.

Bioassay

Kits

Alkaline Phosphatase Detection Kit

MT1000 2-8°C ◆ WET ICE — 1 kit

StarBright® Green Substrate is a fluorogenic substrate specific for the detection of alkaline phosphatase-conjugated secondary systems. Alkaline phosphatase (AP) cleaves the phosphate group of the non-fluorescent StarBright Green Substrate resulting in an intense fluoroscent signal which has an optimal excitation wavelength of 440-450 nm and emission maximum of 505 nm. The large difference between the emission wavelength and the excitation wavelength, also known as Stoke's shift, results in lower levels of background fluorescence and higher detection sensitivity.

StarBright Green Substrate exhibits the following characteristics:
- Low molecular weight (approximately 500 daltons)
- High quantum yield
- Large Stoke's shift (55-65 nm)
- High photostability with no photobleaching
- High water solubility and reconstituted stability
- Expected detection limits of 2 x 10^{-7} units or 1 x 10^{-18} moles of alkaline phosphatase.

Alkaline Phosphatase Detection Kit, Fluorescence

APF -20°C ◆ WET ICE NEW — 1 kit

The kit contains buffers, substrate and control enzyme for an easy and rapid alkaline-phosphatase reporter gene activity assay.
The assay used is fluorometric and therefore is 10 -100 more sensitive than the colorimetric measurement. It is linear over a wide range of enzyme concentrations, which makes it particularly well suited for comparative analysis.
The reporter gene, encoding for alkaline-phosphatase, offers great advantages:
- The enzyme is highly stable.
- The assay is safe and easy to use. It provides rapid and reproducible results.
- When using the mutated version of the human placental alkaline phosphatase (SEAP) which is secreted out of the cells, enzyme activity in the same cell sample can be measured nondestructively and repeatedly over time using an aliquot of the culture medium. This saves the time required for cell extract preparations.

200 µl sufficient for 300 reactions
Components:
4-Methylumbelliferyl phosphate disodium salt,
Fluorescent assay buffer,
Magnesium chloride solution,
Control enzyme,