11 - BioCell gold conjugates

Colloidal gold was first introduced as a cytochemical marker for TEM in 1971 by Faulk and Taylor*, and has become the universally accepted label of choice for electron microscope immunocytochemistry applications and detection of cellular and subcellular macromolecules. It is now a well established technique. Gold labels combine the benefit of unequivocal identification of antigens together with high resolution localisation. The wide choice of gold particle sizes available for labelling at different working magnifications, together with its quantitative facility, also make gold labelling a very flexible tool. One of the major advantages of colloidal gold for electron microscopy is its high electron density, offering easy detection. Procedures for using gold reagents are now well established, and these techniques have been proven to be simple, efficient and economical, as well as non-hazardous.

Colloidal gold has also been established as an excellent marker for light microscopic detection, and silver enhancing methods have allowed gold labels to provide a highly specific and sensitive method for visualising antigens in light microscopy. Gold labels have also become recognised as very important tools for detection and quantitation of proteins, antigens and nucleic acids when used with other techniques such as blotting.

British BioCell International (BBI) is a leading manufacturer of high quality immunogold and silver reagents for research and diagnostic applications, with over 30 years of experience in the manufacture of superior quality nanoparticles and secondary gold probes for the life science industry.

All products are manufactured to the highest specifications, and a quality assurance certificate is provided with each product indicating the mean particle size and coefficient of variation. This section includes many new additions to the BBI product range, such as high quality silver colloids, 2 nm gold conjugates, specialist gold and silver nanoparticles, BIOBOND™ adhesive and BIOMOUNT™ mounting medium.

BBI standard gold conjugates are supplied in a buffer containing 20 % glycerol, and are stable at 4 °C for at least 12 months from delivery, and for many years at -25 °C or below. Antibody gold conjugates are absorbed against certain species of serum proteins, minimising cross reactions to those species.

Detailed instructions for the use of all gold conjugates and silver reagents are given in the technical information booklet provided with each product.

* Faulk, W.P. & Taylor, G.M. *Immunocytochem 8,* 1081-1083, 1991.

Unconjugated gold and silver colloids

Nanometre sized gold and silver particles of uniform shape and size are invaluable tools in nanotechnology, eg. assembled arrays, light scattering etc.

The colloids can be conjugated to antibodies, proteins or many types of macromolecules for binding and reaction labelling studies. The colloids are manufactured with a very narrow size distribution.

Colloidal gold is available in sizes ranging from 2 - 250 nm: 2 - 20 nm optimised for electron microscopy; 30 - 80 nm optimised for lateral flow *in vitro* diagnostics and 100 - 250 nm for optical microscopy.

The silver colloids are available in size range 20 - 80 nm. These are not as well characterised as the gold but give alternative optical properties.

Gold colloids

Particle size (nm)	Particles per ml	Quantity 100 ml	500 ml	20 ml
2	1.5×10^{14}	**R14076**	**R14076-1**	**R14076-2**
5	5.0×10^{13}	**R14077**	**R14077-1**	**R14077-2**
10	5.7×10^{12}	**R14078**	**R14078-1**	**R14078-2**
15	1.4×10^{12}	**R14079**	**R14079-1**	**R14079-2**
20	7.0×10^{11}	**R14080**	**R14080-1**	**R14080-2**
30	2.0×10^{11}	**R14081**	**R14081-1**	**R14081-2**
40	9.0×10^{10}	**R14082**	**R14082-1**	**R14082-2**
50	4.5×10^{10}	**R14150**	**R14150-1**	**R14150-2**
60	2.6×10^{10}	**R14151**	**R14151-1**	**R14151-2**
80	1.1×10^{10}	**R14152**	**R14152-1**	**R14152-2**
100	5.6×10^{9}	**R14153**	**R14153-1**	**R14153-2**
150	1.7×10^{9}	**R14154**	**R14154-1**	**R14154-2**
200	7.0×10^{8}	**R14155**	**R14155-1**	**R14155-2**
250	3.6×10^{8}	**R14156**	**R14156-1**	**R14156-2**

Silver colloids

Particle size (nm)	Particles per ml	Quantity 100 ml	500 ml	20 ml
20	7.0×10^{11}	**R14270**	**R14270-1**	**R14270-2**
40	9.0×10^{10}	**R14271**	**R14271-1**	**R14271-2**
60	2.6×10^{10}	**R14272**	**R14272-1**	**R14272-2**
80	1.1×10^{10}	**R14273**	**R14273-1**	**R14273-2**

All gold colloids are supplied at optical density 1.0 measured at 520 nm. They contain 0.01 % concentration of HAuCl, except 2 nm size which has a concentration of 0.002 %.

Gold conjugates for EM

Gold labels are the most satisfactory method for labelling antigens for visualisation in the electron microscope. Since their introduction, there has been a vast growth in the number of applications in both animal and plant biology. The main advantages of gold labelling lie in the high contrast, the unequivocal nature of the label, the range of particles sizes for different magnifications and the sensitivity and stability of the signal. Minimal particle clustering is also an important feature, especially where quantitative results are required.

EM grade conjugates are available in a range of sizes. All sizes are completely non-overlapping, with the narrowest available coefficient of variation to allow multi-labelling experiments. For low magnification work, larger particles (15 - 30 nm) are more easily seen. Small gold particles (5 - 10 nm) are well suited to high magnification studies and high intensity labelling. 10 nm gold conjugates are recommended for those just beginning immunogold labelling with EM and performing studies over a range of magnifications, whereas 2 nm gold particles offer ultra high sensitivity, but are usually visualised only after silver enhancing on the section.

Conjugated protein	Gold particle size (nm)	Quantity 1 ml	Quantity 0.25 ml
Goat anti-rabbit IgG	2	**R14265**	**R14265-1**
(H+L) (human abs)	5	**R14001**	**R14001-1**
Used in immunocytochemistry of rabbit IgG primary	10	**R14002**	**R14002-1**
	15	**R14003**	**R14003-1**
	20	**R14004**	**R14004-1**
	30	**R14005**	**R14005-1**
	40	**R14160**	**R14160-1**
Goat anti-mouse IgG	2	**R14266**	**R14266-1**
(H) (human abs)	5	**R14008**	**R14008-1**
Used in immunocytochemistry of mouse IgG primary	10	**R14009**	**R14009-1**
	15	**R14010**	**R14010-1**
	20	**R14011**	**R14011-1**
	30	**R14012**	**R14012-1**
Goat anti-mouse IgG	5	**R14162**	**R14162-1**
(H+L) (human abs)	10	**R14163**	**R14163-1**
For use with mouse IgG primary	15	**R14164**	**R14164-1**
	20	**R14165**	**R14165-1**
	30	**R14166**	**R14166-1**
	40	**R14167**	**R14167-1**
Goat anti-mouse IgM	5	**R14013**	**R14013-1**
(mu chain specific)	10	**R14014**	**R14014-1**
Used in immunocytochemistry of mouse IgM primary	15	**R14015**	**R14015-1**
	20	**R14016**	**R14016-1**
	30	**R14017**	**R14017-1**
Goat anti-mouse IgG+IgM	5	**R14018**	**R14018-1**
(H+L) (human abs)	10	**R14019**	**R14019-1**
Used in immunocytochemistry of combined mouse IgG and IgM primary	15	**R14020**	**R14020-1**
	20	**R14021**	**R14021-1**
	30	**R14172**	**R14172-1**

Conjugated protein	Gold particle size (nm)	Quantity 1 ml	Quantity 0.25 ml
Goat anti-mouse IgA	10	**R14173**	**R14173-1**
Goat anti-mouse IgA+IgM+IgG (polyvalent) *Used in immunocytochemistry of combined mouse IgA, IgG and IgM primary*	10	**R14174**	**R14174-1**
Goat anti-mouse IgG (rat abs) *Controls cross reaction. Used in immunocytochemistry of mouse IgG primary*	5	**R14176**	**R14176-1**
Goat anti-rat IgG	5	**R14024**	**R14024-1**
(H+L) (human abs)	10	**R14025**	**R14025-1**
Used in immunocytochemistry of rat IgG primary	15	**R14026**	**R14026-1**
	20	**R14027**	**R14027-1**
Goat anti-rat IgG	5	**R14182**	**R14182-1**
(H+L) (mouse abs)	10	**R14183**	**R14183-1**
Controls cross reaction used in immunocytochemistry of rat IgG primary			
Goat anti-human IgG	10	**R14029**	**R14029-1**
(gamma chain specific) (mouse abs)	15	**R14030**	**R14030-1**
Controls cross reaction. Used in immunocytochemistry of human IgG primary	20	**R14031**	**R14031-1**
Goat anti-human IgG	5	**R14191**	**R14191-1**
(H+L)	10	**R14192**	**R14192-1**
Used in immunocytochemistry of human IgG primary	15	**R14193**	**R14193-1**
	20	**R14194**	**R14194-1**

Gold conjugates for EM

Conjugated protein	Gold particle size (nm)	Quantity 1 ml	Quantity 0.25 ml
Goat anti-human IgM	5	**R14198**	**R14198-1**
(mu chain specific)	10	**R14199**	**R14199-1**
Used in immunocytochemistry of human IgM primary			
Goat anti-guinea pig IgG	5	**R14032**	**R14032-1**
(H+L)	10	**R14033**	**R14033-1**
Used in immunocytochemistry of guinea pig IgG primary	15	**R14034**	**R14034-1**
	20	**R14035**	**R14035-1**
Rabbit anti-chicken IgG	5	**R14203**	**R14203-1**
(H+L)	10	**R14204**	**R14204-1**
Used in immunocytochemistry of chicken IgY and IgG primary			
Rabbit anti-goat IgG	5	**R14040**	**R14040-1**
(H+L)	10	**R14041**	**R14041-1**
Used in immunocytochemistry of goat IgG primary	15	**R14042**	**R14042-1**
	20	**R14043**	**R14043-1**
Rabbit anti-goat IgG	5	**R14213**	**R14213-1**
(H+L) (human abs)	10	**R14214**	**R14214-1**
For use with goat IgG primary			
Donkey anti-sheep IgG	5	**R14044**	**R14044-1**
(H+L)	10	**R14045**	**R14045-1**
Used in immunocytochemistry of sheep IgG primary	15	**R14046**	**R14046-1**
	20	**R14047**	**R14047-1**
Goat anti-horseradish peroxidase	5	**R14218**	**R14218-1**
	10	**R14219**	**R14219-1**
Used as a secondary corroborative probe			
Protein A	5	**R14048**	**R14048-1**
Mimics a secondary antibody by binding to the FC part of the primary	10	**R14049**	**R14049-1**
	15	**R14050**	**R14050-1**
	20	**R14051**	**R14051-1**
Protein G	5	**R14052**	**R14052-1**
Mimics a secondary antibody by binding to the FC part of the primary	10	**R14053**	**R14053-1**
	15	**R14054**	**R14054-1**
	20	**R14055**	**R14055-1**

Conjugated protein	Gold particle size (nm)	Quantity 1 ml	Quantity 0.25 ml
Protein AG	5	**R14225**	**R14225-1**
Mimics a secondary antibody by binding to the FC part of the primary	10	**R14226**	**R14226-1**
	15	**R14227**	**R14227-1**
	20	**R14228**	**R14228-1**
Cationic colloidal gold	5	**R14060**	**R14060-1**
(poly-L-lysine)	10	**R14061**	**R14061-1**
Allows staining of negatively charged sites in cells and tissue	15	**R14062**	**R14062-1**
	20	**R14063**	**R14063-1**
Bovine serum albumin	5	**R14064**	**R14064-1**
Used as a negative control	10	**R14065**	**R14065-1**
	15	**R14066**	**R14066-1**
	20	**R14067**	**R14067-1**
Goat F(ab')2 anti-rabbit IgG	5	**R14006**	**R14006-1**
(H+L) (human abs)	10	**R14007**	**R14007-1**
Use of F(ab')2 fractions can reduce background			
Goat F(ab')2 anti-mouse IgG	5	**R14248**	**R14248-1**
(H)	10	**R14249**	**R14249-1**
Use of F(ab')2 fractions can reduce background with mouse IgG primary			
Goat F(ab')2 anti-mouse IgM	5	**R14252**	**R14252-1**
(mu chain specific)	10	**R14253**	**R14253-1**
Use of F(ab')2 fractions can reduce background with mouse IgM primary			
Goat F(ab')2 anti-mouse IgG+IgM	5	**R14022**	**R14022-1**
(H+L) (human abs)	10	**R14023**	**R14023-1**
Use of F(ab')2 fractions can reduce background with mouse IgG and IgM primary			

(H) heavy chain specific

(H+L) heavy and light chain specific

(human abs) absorbed against human serum proteins

(mouse abs) absorbed against mouse serum proteins

(rat abs) absorbed against rat serum proteins

Gold conjugates for LM

Light microscope immunocytochemistry with gold conjugates follows similar principles to the well established indirect labelling methods using peroxidase labelled secondary antibodies, but offers several advantages. No hazardous materials are involved, the procedure is simpler and yields a much more intense stain than conventional peroxidise and PAP techniques. The natural red colour of the gold in transmitted light gives a stain which is easily visualised. Of particular importance is the ability to label sections from the same block for both light and electron microscopy.

Gold conjugate particles are available in 2 nm and 5 nm particle diameters and produce excellent labelling in wax, resin embedded or frozen sections. With silver enhancement a dense black reaction is produced which is clearly localised and can be counterstained with a variety of tissue stains.

Conjugated protein	Gold particle size (nm)	Quantity 1 ml	Quantity 0.25 ml
Goat anti-rabbit IgG (H+L) (human abs)	5	**R14085**	**R14085-1**
Goat anti-mouse IgG (H) (human abs)	5	**R14087**	**R14087-1**
Goat anti-mouse IgG (H+L) (human abs)	5	**R14168**	**R14168-1**
Goat anti-mouse IgM (mu chain specific)	5	**R14088**	**R14088-1**
Goat anti-mouse IgG+IgM (H+L) (human abs)	5	**R14089**	**R14089-1**
Goat anti-mouse IgG (rat abs)	5	**R14178**	**R14178-1**
Goat anti-rat IgG (H+L) (human abs)	5	**R14091**	**R14091-1**
Goat anti-rat IgG (H+L) (mouse abs)	5	**R14184**	**R14184-1**
Goat anti-human IgG gamma chain specific	5	**R14092**	**R14092-1**
Goat anti-human IgG (H+L)	5	**R14196**	**R14196-1**
Goat anti-human IgM (mu chain specific)	5	**R14200**	**R14200-1**
Goat anti-guinea pig IgG (H+L)	5	**R14093**	**R14093-1**
Rabbit anti-chicken IgG (H+L)	5	**R14205**	**R14205-1**

Conjugated protein	Gold particle size (nm)	Quantity 1 ml	Quantity 0.25 ml
Rabbit anti-goat IgG (H+L)	5	**R14095**	**R14095-1**
Rabbit anti-goat IgG (H+L) (human abs)	5	**R14215**	**R14215-1**
Donkey anti-sheep IgG (H+L)	5	**R14096**	**R14096-1**
Goat anti-horseradish peroxidase	5	**R14220**	**R14220-1**
Protein A	5	**R14097**	**R14097-1**
Protein G	5	**R14098**	**R14098-1**
Protein AG	5	**R14229**	**R14229-1**
Cationic colloidal gold (poly-L-lysine)	5	**R14100**	**R14100-1**
Bovine serum albumin (negative control)	5	**R14101**	**R14101-1**
Goat F(ab')2 anti-rabbit IgG (H+L) (human abs)	5	**R14086**	**R14086-1**
Goat F(ab')2 anti-mouse IgG (H) (human abs)	5	**R14250**	**R14250-1**
Goat F(ab')2 anti-mouse IgM (mu chain specific)	5	**R14254**	**R14254-1**
Goat F(ab')2 anti-mouse IgG+IgM (H+L) (human abs)	5	**R14090**	**R14090-1**

Gold conjugates for *in situ* hybridisation

Specific DNA or RNA fragments may be readily identified within cells after *in situ* hybridisation with appropriate DNA/RNA probes. During hybridisation at the cellular level, the labelled probe binds to complementary cellular target DNA/RNA, then is detected with a suitably labelled antibody. Immunogold techniques have become popular for both light and electron microscope detection of DNA/RNA in cells, due to their sensitivity and high resolution. Tissue sections can be counterstained with all normal stains.

Conjugated protein	Gold particle size (nm)	Quantity 1 ml	Quantity 0.25 ml
Streptavidin	2 EM grade	**R14280**	**R14280-1**
For the indirect detection of a biotinylated primary antibody	5 EM grade	**R14056**	**R14056-1**
	10 EM grade	**R14057**	**R14057-1**
	15 EM grade	**R14058**	**R14058-1**
	5 LM grade	**R14099**	**R14099-1**
Goat anti-biotin	5 EM grade	**R14036**	**R14036-1**
For the indirect detection of a biotinylated primary antibody	10 EM grade	**R14037**	**R14037-1**
	15 EM grade	**R14038**	**R14038-1**
	20 EM grade	**R14039**	**R14039-1**
	5 LM grade	**R14094**	**R14094-1**
Monoclonal anti-biotin	2 EM grade	**R14281**	**R14281-1**
For the indirect detection of a biotinylated primary antibody			

2 nm gold conjugates

These ultra small gold conjugates may be used effectively for EM, LM and immunoblotting applications. A major advantage of the ultra small gold particles is the increased penetration of antibodies into cells and tissues without the need for permeabilisation. Together with silver enhancing techniques they provide an extremely sensitive low background labelling with increased penetration into tissue sections and through cell membranes.

Specialist gold and silver nanoparticles

R14282 Raman specification gold colloid. 150 µl
R14283 Raman specification silver colloid. 150 µl

Customised conjugation service

A customised conjugation service is available, offering bespoke production of high quality conjugates optimised for your sensitivity specifications. Antibodies, proteins and macromolecules can be labelled with gold particles of all sizes.

Please contact us with your requirements.

Gold conjugates for blotted proteins

Blotting techniques have been established for a number of years, and the use of immunogold conjugates provides a safe, sensitive and economical alternative to traditional labels.

Gold conjugates are an extremely sensitive method for identification of immobilised proteins and nucleic acids on membranes such as nitrocellulose or PVDF. This technique can be used for determination of protein bands transferred from electrophoretic gels, DNA fragments hybridised on membranes with nucleic acid probes, and identification and quantitation of proteins and macromolecules blotted directly in small quantities.

Blotting grade gold conjugates are available with either 2 or 20 nm gold particles.

Conjugated protein	Gold particle size (nm)	Quantity 1 ml	Quantity 2 ml
Goat anti-rabbit IgG (H+L) (human abs)	20	**R14102**	**R14102-2**
Goat anti-mouse IgG (H) (human abs)	20	**R14103**	**R14103-2**
Goat anti-mouse IgG (H+L) (human abs)	20	**R14269**	**R14269-2**
Goat anti-mouse IgM (mu chain specific)	20	**R14104**	**R14104-2**
Goat anti-mouse IgG+IgM (H+L) (human abs)	20	**R14105**	**R14105-2**
Goat anti-rat IgG (H+L) (human abs)	20	**R14106**	**R14106-2**
Goat anti-human IgG (gamma chain specific)	20	**R14107**	**R14107-2**
Goat anti-human IgG (H+L)	20	**R14268**	**R14268-2**
Goat anti-guinea pig IgG (H+L)	20	**R14108**	**R14108-2**
Goat anti-biotin	20	**R14109**	**R14109-2**
Rabbit anti-goat IgG (H+L)	20	**R14110**	**R14110-2**
Donkey anti-sheep IgG (H+L)	20	**R14111**	**R14111-2**
Protein A	20	**R14112**	**R14112-2**
Protein G	20	**R14113**	**R14113-2**
Protein AG	20	**R14267**	**R14267-2**
Streptavidin	20	**R14114**	**R14114-2**
Cationic colloidal gold (poly-L-lysine)	20	**R14232**	**R14232-2**
Bovine serum albumin	20	**R14245**	**R14245-2**

PROTOGOLD®

PROTOGOLD colloidal gold solution has been specifically developed to give intense staining of proteins blotted onto membranes by dot blots or electrophoretic gels. The gold particles are negatively charged and bind selectively to the blotted proteins with very low background signal, producing a dark red stain. The gold signal does not fade and, when used in conjunction with the BL silver enhancing kit, a further amplification of the signal by x10 - 100 can be achieved. This method is more sensitive than Coomassie Blue or silver staining, and the kit will stain over 20 blots with high intensity.

Benefits of using PROTOGOLD include fast quantitative protein determination, with visible staining of the proteins occurring within minutes; greater sensitivity, allowing detection of picogram quantities of protein; and high resolution, offering crisp images. It is easy to use, with no destaining necessary, allowing staining to be continued indefinitely without over staining.

R14084 PROTOGOLD kit. 500 ml
R14257 Test strips. 50

Silver enhancing kits

These kits offer ease of use and sensitivity for the amplification of immunogold labels in electron and light microscopy and blotting applications. Enhancement occurs during the reduction of silver from one solution (the enhancer) by another (the initiator) in the presence of gold particles. This reduction causes silver to build up on the surface of gold particles, with no diffusion of the signal due to the discrete nature of the silver growth on the gold particles. Enhancement is rapid and easily controllable; the reaction can be monitored in bright light and terminated by washing in tap water, with signal amplifications of x10 - 100 readily achieved.

Two silver enhancing kits are available for different applications, an EM/LM kit and a blotting kit.

R14115 LM and EM kit*. 2 x 15 ml
R14116 Blotting kit**. 2 x 250 ml
R14117 Test strips. 10

* Sufficient for 300 slides
** Sufficient for 20 - 30 blots

Blocking reagents

Non-specific labelling of specimens can occur during immunolabelling procedures. The source of this background labelling must be determined by careful and systematic use of controls, and eliminated to allow proper analysis of the specimen. Background labelling can occur either in the specimen or in the incubating solutions and in either case the background can be substantially reduced by the careful use of blocking reagents.

R14120 Tween® 20. 10 ml
R14121 BSA (powder) (fatty acid free). 10 g
R14122 Gelatin (fish) (45 %). 10 ml

Biomount™

Biomount mounting medium reduces fading of immunogold/silver signals in sections on glass slides, and can be used with both resin and wax embedded tissue sections. It is miscible with xylene, and may be applied following dehydration. Labelling will retain its intensity and contrast.

R1354 Biomount. 100 ml *Flammable, harmful*

BioBond™

For light and electron microscopy the choice of specimen preparation is critical for the preservation of antigens in the sample. Having prepared the tissue sample for immunolabelling it is imperative to perform the incubation to maximise the specific signal and minimise the background.

Under some incubation conditions tissue sections can be removed from the slide. Some adhesives are not suitable for use with immunogold labelling because of the increased background caused by the attraction of gold particles to the adhesive on the slide.

BioBond is a special adhesive which gives a good bonding between the slide and tissue section for subsequent incubation. It is particularly effective for use with severe incubation conditions such as those used for *in situ* hybridisation. It is suitable for many types of tissue specimen including paraffin wax or resin sections, cell smears, cytospin or cryostat sections.

Sufficient to coat at least 1000 slides.

R1355 BioBond tissue adhesive. 20 ml