FT-51558C

(Strept)avidin reagents

Description

Uptima (Strept)avidin conjugates are high quality reagents to be used with biotinylated probes (notably antibodies) in various immunotechnologies (ELISA, Blotting, Cytometry, Immunohistology).

	Unlabeled	Fluorescein (FITC)	Peroxidase (HRP)	Alkaline Phosphatase	R-Phycoerythrin
Streptavidin Conjugates					
	UP51558B, 2mg UP51558C, 5mg UP51558, bulk	UP277130, 1 mg	UP395888, 1mg	UP518498, 1mg	Inquire
Neutralized Avidin Conjugates					
	UP25527A, 5mg UP25527B, 10mg	FP-73578A, 1 mg	UP36570A, 1mg	UP38592A, 1mg	UP31259A, 1mg
Avidin Conjugates					
	UP39860D, 5mg UP39860, bulk	Inquire	Inquire	Inquire	Inquire

Form: powder (unlabeled), or ready-to-use solution (labeled Streptavidins)

Storage: +4°C (L) (long term storage: -20°C for unlabeled products)
Shipping at room temperature (with blue ice for SAV-RPE)

Associated Products:
Streptavidin Magnetic Beads *Uptibeads,* (UPR09030, UPR09031, UPR09020, UPR09021)
Imobilized biotins (FT-UP88722) and avidins (FT-UP29337); Polymeric Streptavidin-RP (FT-CV3681)

Scientific and Technical Information

Both Streptavidin and NeutralizedAvidin are offered labeled by enzymes and fluorophores for ELISA, blotting, FCM, and IH techniques. Uptima (Strept)avidin conjugates are of very high quality, and offer advantages when compared with directly labeled primary antibodies :

- Lower background
- Amplified detection signals
- Easier to calibrate than different primary or secondary antibodies
- More convenient for rarely used antibodies
- Increased flexibility

- **Streptavidin** is isolated from *Streptomyces avidinii*, and has a very high affinity for biotin (>10^{-14} M^{-1}). This makes the streptavidin-biotin interaction an ideal tool for many research applications. Streptavidin does not have any carbohydrates and has a lower ionic charge than avidin, resulting in a lower non-specific background. This makes streptavidin a preferred choice for many biotin-based applications.

- **Avidin** is purified from eggs and has a even higher affinity for biotin (>10^{-15} M^{-1}). It does not contain the RYD sequence found in Streptavidin, that is homologous of some integrins, giving the advantage of no unspecific binding that is observed in some detection systems with Streptavidin. Avidin presents however glycones and a higher ionic net charge, that generated in some application higher background. Uptima offers to that point a chemically modified avidin, gathering the advantage of high specificity and affinity of avidin, but lowest background like Streptavidin. This **neutralized avidin** gives unsurpassed detection of biotinylated molecules.

- Labels: **HRP Peroxidase, AP Phosphatase, FITC, R-PE**

Horseradish peroxidase (HRP) is selected for its high activity and conjugated to the antibodies following an optimized process, which results in highly sensitive and stable antibodies. Peroxidase is one of the most commonly used enzymes as it is cheap and versatile, with an extensive range of soluble and insoluble substrates available. Recommended colorimetric substrates for HRP are TMB for ELISA (cat #UP664780) and TMB for blotting (cat # UP15426D). Higher sensitivity can be achieved by using a chemiluminescent substrate (UptiLight #UP99619A). One of the primary problems associated with HRP is non- specific staining that results form endogenous peroxidase activity within immunocytochemistry applications.
Applications: blotting, immunohistochemistry or ELISA
(Strept)Avidin-AP:
Working dilution for immunohistology is approximately 1:1000. However, each investigator should determine the optimal working dilution for each specific research application. Dilutions up 1:100 000 can be used with ECL reagents (see UptiLight).

Alkaline Phosphatase (AP) is an enzyme, which is isolated from calf intestines. It gives a more linear activity than peroxidase, and is suitable for most immunodetections. Alkaline phosphatase is especially recommended for applications, where high levels of endogenous peroxidase are present. Because reaction rates remain linear when using AP, just allowing the reaction to proceed for longer periods of time can increase the sensitivity. Recommended substrates for alkaline phosphatase are: BCIP/NBT for blotting and immunohistochemical applications (cat # UP096051) and pNPP for ELISA (cat # UP732500). Endogenous alkaline phosphatase activity found in some samples can be inhibited by levamisole. The reaction with pNPP allows kinetic readings.
Applications : blotting, immunohistochemistry or ELISA
(Strept)Avidin-AP:
Working dilution for immunohistology is approximately 1:1000. However, each investigator should determine the optimal working dilution for each specific research application.

Fluorescein (FITC) is a commonly used fluorescent label with an excitation wavelength of 495nm (argon laser)(max at 491nm), and an emission at 528nm (max at 518nm). Uptima FITC labeled antibodies are conjugated with 4-8 fluorophores per molecule to achieve the best signal to noise ratio.
Applications : flow cytometry or immunohistochemistry (Vigier at all, 1988)
(Strept)Avidin-FITC:
Suggested working concentration : one microgram to stain 1.0 X 10^6 cells in flow cytometric applications. However, each investigator should determine their own optimal working dilution for each specific research application

R-PhycoErythrin (RPE) elicit exceptional fluorescent properties for labeling techniques, especially when high sensitivity or multicolor detection are required. It has 1/Broad and high absorption of light suitable to many light sources 2/Very intense emission of light, thanks to highest quantum yield 3/Relative large Stokes shifts allowing simultaneous use with FITC or TR, and any other equivalent fluorochromes 4/Very high water solubility.
(Strept)Avidin-RPE
R-phycoerythrin has an absorbency maximum at 565.5nm and an emission maximum at 578nm.
R-phycoerythrin to avidin molar ratio is between 0.7 and 1.3.
Suggested concentration of use is approximately 0.2-0.5 micrograms of product UP31259 to stain 1.0 X 10^6 cells in flow cytometry applications. However, each investigator should determine the optimal working dilution for each specific research application

Use

- **Unlabeled (strept)avidins** are classically used for:

-coating of microplates and other supports
-creating conjugates (of peptides, antibodies, any biological biomolecules)

Guidelines for coating protocols: 0.1 to 20µg/ml concentration in 0.1M carbonate pH9.6 or any other suitable buffer is recommended depending on application.

- **Labeled Streptavidin and NeutralizedAvidin** are classically used for:

-direct immunodetection of bound biotin (ELISA sandwich)
-indirect detection of biotin (ELISA inhibition)

Guidelines for detection protocols: PBST (150mM NaCl, 20mM phosphate, 0.05% Tween20) is a good buffer for most applications. However, TBS (150mM NaCl, 20mM Tris, pH7.5) is recommended for alkaline phosphatase-conjugates. A saturating agent may also be added. The dilution of use should be determined in each detection technique (ELISA or Blotting, FCM or IH…) and application, depending on assay conditions (saturating agent, nature of enzymatic substrate, duration of incubation…). 1/1000-1/50000 dilutions ordinarily suits for chromogenic substrates (TMB #UP66478, pNPP #UP66479…), and up 1/100 000 for chemiluminescent substrates (UptiLight #UP99619). Suggested dilutions may be given in batch certificates.

Reconstitution of freeze-dried products

Reconstitute in 1 ml d. water. Centrifuge product if the solution is not clear after reconstitution. To judge clarity, draw product in the clean Pasteur pipette. Storing the product for more than one day at final working dilutions is not recommended. For extended storage as a liquid, add an equal volume of glycerol (ACS or better grade) for a final concentration of 50% followed by storage at –20°C. please note that the concentration of protein and buffer salts will be one half of the original after the addition of glycerol. Alternatively, the product may be aliquoted and frozen at –70°C or below, in the absence of glycerol. It's to avoid repeated freezing and thawing. Expiration date: One year from date of reconstitution

Working dilutions of approximately 1:4000 for protein blotting, 1:1000 for immunohistology and 1:20 000 for ELISA. However, each investigator should determine their own optimal working dilution for each specific research application.

Other information

For use *in vitro* only, not for diagnostic.

For any information, please contact Uptima, or your local distributor.
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Associated products and documents:

Biotinylated secondary antibodies (p.A324)
Other (strept)aviding reagents (p.A350)
chromogenic substrates for HRP (UptiLight ECL substrates, TMB #UP664781), for AP (pNPP #UP664791)

Other immunologicals for ELISA (p.A361), Blotting (p.A368), IHC/IF (p.A377), MicroArray and FCM (p.A396):
including buffers and saturants (p.A365):
TBS buffer (Tris Buffer Saline) UP74004A (also available with non fat milk #GS4160, with Tween20 #GS4200,...)
PBS buffer (Phosphate Buffer Saline) #UP68723A (also available with non fat milk #GS4180, with Tween20 #GS4250,...)
BSA #UPQ84170 (powder) or #UP900130 (soln 30%)
Saturating agents: SeaBlock #UP40301A, Non fat milk #768701, BioBlock #N13650

Literature:

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