

Datasheet for S000-04**Streptavidin Cy3 Conjugated****Overview**

Description:	Streptavidin CY3 Conjugated - S000-04
Item No.:	S000-04
Size:	1 mg
Applications:	Dot Blot, IF, IHC, Multiplex

Product Details

Background:	Streptavidin is isolated from bacteria, <i>Streptomyces avidinii</i> , and has an exceptionally high binding affinity for B7 (biotin). Rockland offers streptavidin in unconjugated and conjugated forms for common immunoassays including ELISA, western blotting, immunohistochemistry. Streptavidin is a tetrameric protein capable of binding 4 biotin groups to each molecule of streptavidin. While streptavidin has identical binding properties as avidin, it lacks the glycoprotein portion of the molecule and therefore shows less non-specific binding. Streptavidin is a slightly smaller molecule with a molecular weight of approximately 53.6 kDa. The sequence of avidin only shows 30% homology with streptavidin, and anti-avidin and anti-streptavidin antibodies are not immunologically cross reactive. Rockland conjugates a broad group of secondary antibodies to many of the classic fluorescent markers including fluorescein, rhodamine, Texas Red, CyDyes™ and Phycoerythrin (RPE). All of the conjugates are ideal for various immunofluorescence based assays including fluorescent western blotting, immunofluorescence microscopy, FLISA, and more. Rockland also produces many next generation fluorochrome dyes designed for detection of primary antibodies in multiplex, multi-color analysis.
Synonyms:	SA, S avidin, streptococcus avidin, Streptavidin Cy3 Conjugated
Conjugate:	Cy3™
F/P Ratio:	4.1

Target Details

Purity/Specificity:	This product was prepared from chromatographically pure Streptavidin. Assay by immuno-electrophoresis resulted in a single precipitin arc against anti-Streptavidin.
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Application Details

Tested Applications:	Dot Blot
Suggested Applications:	IF, IHC, Multiplex (Based on references)
Application Note:	Streptavidin CY3 conjugated has been tested by dot blot and is designed for immunofluorescence microscopy, fluorescence based plate assays (FLISA) and fluorescent western blotting. This product is also suitable for multiplex analysis, including multicolor imaging, utilizing various commercial platforms.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
FC:	1:500 - 1:2,500
FLISA:	1:10,000 - 1:50,000
IF:	1:1,000 - 1:5,000

Formulation

Physical State:	Lyophilized
Concentration:	1.0 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	10 mg/mL Bovine Serum Albumin (BSA) - Immunoglobulin and Protease free
Reconstitution Volume:	1.0 mL
Reconstitution Buffer:	Restore with deionized water (or equivalent)

Shipping & Handling

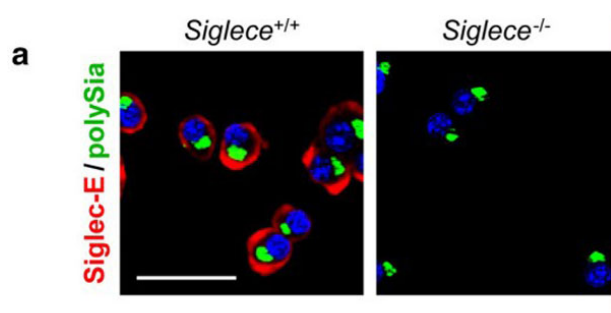
Shipping Condition:	Ambient
Storage Condition:	Store vial at 4° C prior to restoration. For extended storage aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

Images



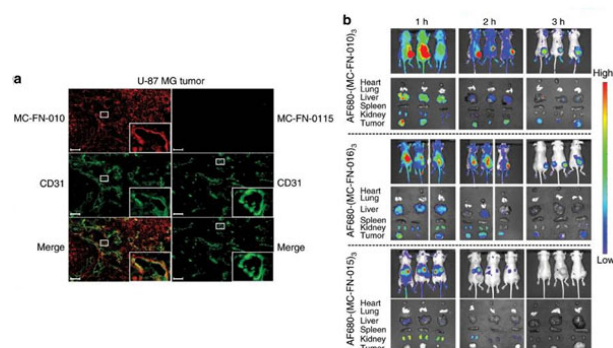
Bottle

Streptavidin CY3 Conjugated



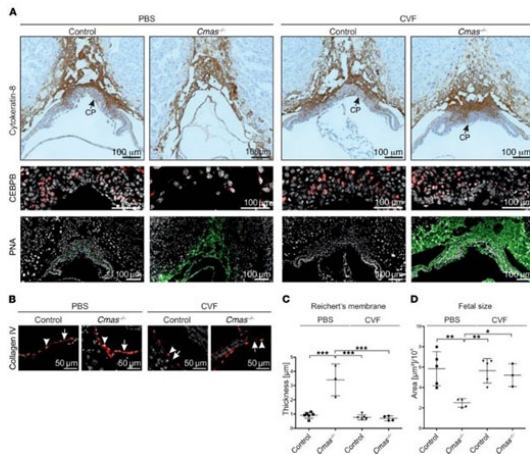
Immunofluorescence Microscopy

Loss of Siglec-E abrogates responsiveness to polySia and enhances LPS-induced activation. **a** Compared to wildtype BV2 cells (Siglec-E^{+/+}), the immunoreactivity of Siglec-E (red), but not polySia (green) is abolished by CRISPR/spCas9-mediated knockout of Siglec-E (Siglec-E^{-/-}, clone D19). Nuclear counterstain with DAPI (blue). Scale bar, 50 μ m. Cells were incubated with primary antibodies and for with HRP-conjugated anti-sheep IgG for the detection of Siglec-E-specific antibody, followed by incubation, then enzymatically produced biotin precipitate was detected by Cy3-conjugated streptavidin (1:1.000 p/n S000-04), before fluorescently labeled secondary antibodies were added. Fig. 6. PMID: 32725371.



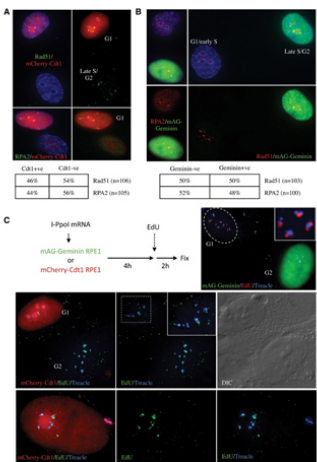
Immunofluorescence Microscopy

Targeting the tumor vasculature with selected cystine-knot miniproteins. a Specific binding of MC-FN-010 to tissue sections derived from the human U-87 MG glioblastoma cell line grown as mouse xenograft tumor. Representative immunofluorescence staining of U-87 MG tumor tissues and normal mouse brain with EDB ligand MC-FN-010 and negative control MC-FN-0115. Tissue sections (6 μ m) were stained with tetramerized cystine-knot miniprotein-biotin/streptavidin-Cy3 complex (red) and an anti-CD31 antibody to visualize vasculature (green). Scale bars, 100 μ m. b In vivo and ex vivo imaging of U-87 MG bearing mice. Tumors derived from human U-87 MG cells injected s.c. in flanks of mice were imaged after i.v. application of 3.34 nmol AF680-(MC-FN-010)3, AF680-(MC-FN-016)3 (EDB binder), and control AF680-(MC-FN-0115)3. Mice (n = 3) were stratified according to their tumor sizes. Fig. 4. PMID: 31941901.



Immunohistochemistry

Maternal decompensation rescues defects in extraembryonic tissues and growth restriction of *Cmas*^{-/-} mice. Pregnant *Cmas*^{+/-} mice were treated at E4.5 and E6.5 either with PBS (n = 3) or CVF (n = 3) to deplete maternal C3. E8.5 sagittal uteri paraffin sections (A and B). (A) The reduced size of the EPC and lack of a CP in *Cmas*^{-/-} embryos of PBS-treated mothers was reverted to the control phenotype upon CVF treatment, as visualized by immunohistochemical cytokeratin-8 staining. The lack of CEBPB reactivity (indirect immunofluorescence) in *Cmas*^{-/-} embryos of PBS-treated mothers was restored upon CVF treatment. PNA reactivity documenting the loss of cell surface sialylation was maintained in *Cmas*^{-/-} embryos of PBS- and CVF-treated mothers, indicating that the asialo phenotype was not influenced by CVF. Representative images of experiments with PBS-treated mice: control, n = 5; *Cmas*^{-/-}, n = 3 embryos; CVF treated mice: control, n = 5; *Cmas*^{-/-}, n = 4 embryos. (B) Collagen IV indirect immunofluorescence (red). Thickened RM (arrow) in *Cmas*^{-/-} embryos of PBS-treated mothers was converted to the control phenotype upon CVF treatment. Parietal endoderm is marked by arrowheads. Nuclei shown in white were stained with DAPI. (C) Quantification of RM thickness measured on collagen IV immunofluorescence images at the anti-mesometrial pole (PBS: control, n = 6; *Cmas*^{-/-}, n = 3 embryos; CVF: control, n = 5; *Cmas*^{-/-}, n = 4 embryos). (D) Mean of fetal size as measured by the sum of the areas of the amniotic cavity, exocoelomic cavity, ectoplacental cavity, and embryo proper in (μm²/105) (PBS: control, n = 5; *Cmas*^{-/-}, n = 4 embryos; CVF: control, n = 5; *Cmas*^{-/-}, n = 3 embryos); Statistical analyses by 1-way ANOVA with Newman-Keuls post test (*P < 0.05; **P < 0.01; ***P < 0.001). Error bars indicate SD (C and D). Primary antibodies were applied. Cebpb antibody was preincubated with biotinylated anti-mouse IgG Fab fragments prior to use. Secondary antibodies added. Biotinylation was detected using streptavidin-HRP at 1:500, and the signal was amplified by biotin tyramide. Streptavidin-Cy3, (1:500, p/n S000-04) served as the detection system. Cytokeratin-8 detected using the HRP anti-rat IgG conjugate. Figure 7. PMID: 30382946.



Immunofluorescence Microscopy

HR factors are recruited, and damage-induced DNA synthesis can be observed at I-Ppol-induced nucleolar caps in G1 cells. (A) I-Ppol transfection of RPE1 cells stably expressing FUCCI component mCherry-Cdt1 reveals that HR factors Rad51 and RPA2 are recruited comparably to nucleolar caps in G1 (red) and late S/G2 (nonred) cells. Quantification is shown below. (B) I-Ppol transfection of RPE1 cells stably expressing FUCCI component mAG-Geminin reveals that HR factors Rad51 and RPA2 are recruited comparably to nucleolar caps in G1 (nongreen) and late S/G2 (green) cells. Quantification is shown below. (C) RPE1 cells stably expressing FUCCI component mAG-Geminin or mCherry-Cdt1 were transfected with I-Ppol mRNA; 4 h after transfection, fresh medium containing EdU was added to cells. Following a 2-h pulse of EdU labeling, cells were fixed. Treacle antibodies detected damage-induced nucleolar caps. Damage-induced DNA synthesis was visualized using biotin-azide and fluorophore-conjugated streptavidin (see the Materials and Methods for details). In the top right panel, EdU incorporation can be observed at damage-induced nucleolar caps in G1 (nongreen) mAG-Geminin cells. In the middle panels, EdU incorporation is observed in nucleolar caps in both G1 (red) and G2 (nonred) mCherry-Cdt1 cells. A DIC image is shown at the right. In the bottom panels, a single enlarged G1 cell from the same experiment is shown. Fig 6. PMID: 26019174

References

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