Datasheet for 200-402-095

Streptavidin Antibody Fluorescein Conjugated

Overview

Description:	Anti-Streptavidin (RABBIT) Antibody Fluorescein Conjugated - 200-402-095
Item No.:	200-402-095
Size:	100 µg
Applications:	Dot Blot, WB, Cellular Assay
Reactivity:	Streptavidin
Host Species:	Rabbit

Product Details

Background:	Streptavidin antibody is a 60 kDa protein purified from the bacterium Streptomyces avidinii. Streptavidin homo-tetramers have an extraordinarily high affinity for biotin (also known as vitamin B7). With a dissociation constant (Kd) on the order of ≈10−14 mol/L,[1] the binding of biotin to streptavidin is one of the strongest non-covalent interactions known in nature. Streptavidin is used extensively in molecular biology and bio-nanotechnology due to the streptavidin-biotin complex's resistance to organic solvents, denaturants (e.g. guanidinium chloride), detergents (e.g. SDS, Triton), proteolytic enzymes, and extremes of temperature and pH. Anti-STREPTAVIDIN antibody is ideal for investigators involved in GFP and Epitope research.
Synonyms:	rabbit anti-Streptavidin Antibody FITC Conjugation, Fluorescein conjugated rabbit anti-Streptavidin antibody, Anti-Streptavidin FITC Antibody
Host Species:	Rabbit
Conjugate:	Fluorescein (FITC)
Clonality:	Polyclonal
Format:	IgG
F/P Ratio:	1.94
Specific Activity:	4.72

Target Details

Reactivity:	Streptavidin

Immunogen Type:	Native Protein
Immunogen:	Streptavidin (Streptomyces avidinii)
Purity/Specificity:	Anti-Streptavidin Antibody Fluorescein Conjugated is an IgG fraction antibody purified from monospecific antiserum by a multi-step process which includes delipidation, salt fractionation and ion exchange chromatography followed by extensive dialysis against the buffer stated above. Assay by immunoelectrophoresis resulted in a single precipitin arc against anti-fluorescein, anti-Rabbit Serum and Streptavidin. No reaction was observed against Avidin.

Application Details

Tested Applications:	Dot Blot, WB
Suggested Applications:	Cellular Assay (Based on references)
Application Note:	Anti-Streptavidin Antibody Fluorescein Conjugated has been tested by dot blot and western 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:	User Optimized
FLISA:	User Optimized
IF:	1:500-1:2,500

Formulation

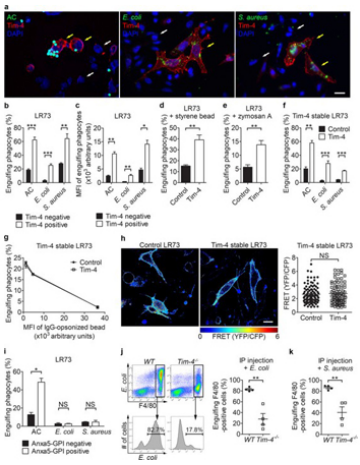
Physical State:	Lyophilized
Concentration:	1 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	10 mg/mL Bovine Serum Albumin (BSA) - Immunoglobulin and Protease free
Reconstitution Volume:	100 µL
Reconstitution Buffer:	Restore with deionized water (or equivalent)

Shipping & Handling

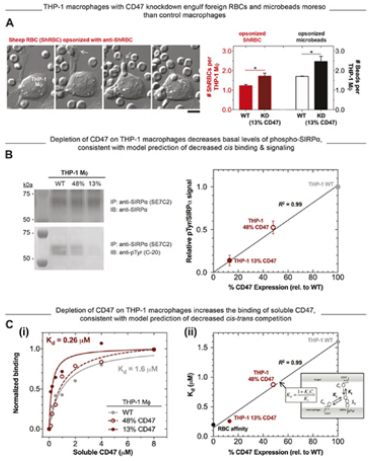
Shipping Condition:	Ambient

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



Flow Cytometry
a–c LR73 cells transfected with HA-Tim-4 were incubated with TAMRA-labeled apoptotic thymocytes, FITC-labeled E. coli, or S. aureus bioparticles for 2 h, extensively washed with ice-cold PBS, stained with anti-HA antibody, and observed using confocal microscopy (a) or flow cytometry (b, c n = 3). TAMRA- or FITC-positive cells were considered to be phagocytes engulfing apoptotic thymocytes, or E. coli or S. aureus bioparticles, respectively. Yellow arrows indicate Tim-4-positive cells, and white arrows indicate Tim-4-negative cells. Scale bar, 10 µm. d, e Phagocytosis of red fluorescence-labeled polystyrene beads (carboxylate-modified beads) (d n = 3) or zymosan A (e n = 3) by LR73 cells transfected with HA-Tim-4 was analyzed as in (b). f LR73 cells stably expressing Tim-4 were incubated with the E. coli or S. aureus bioparticles for 2 h, and engulfing phagocytes were analyzed using flow cytometry (n = 4). g The indicated cells were incubated with FITC-labeled IgG-coated streptavidin beads (p/n 200-4295) for 2 h and analyzed by flow cytometry. h LR73 cells stably expressing Tim-4 or control cells were transfected with Raichu-Rac1, and FRET was measured (99 cells for control-stable cells, 84 cells for Tim-4-stable cells). i Phagocytosis of the indicated targets by LR73 cells transfected with HA-Anxa5-GPI was evaluated as in (b) (n = 3). Scale bar, 10 µm. j, k FITC-labeled E. coli (j) or S. aureus (k) bioparticles were intraperitoneally injected into WT or Tim-4−/− mice, and then, 20 min after injection, the mice were sacrificed, and peritoneal exudates were stained with anti-F4/80 antibody. F4/80- and FITC-positive cells were considered to be phagocytes engulfing E. coli (j four mice per each sample) or S. aureus (k four mice per each sample) particles. All data are shown as the mean ± standard error of mean. Images are representative of three independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001. NS not significant, AC apoptotic cells. Fig 1.
PMID: 32703939



Immunofluorescence Microscopy
Depleting CD47 on macrophages increases engulfment activity, decreases SIRPα signaling and increases SIRPα affinity. (A) Stable knockdown of CD47 on THP-1 macrophages to 48% or 13% of wild-type (WT) levels was used to phenocopy anti-CD47 effects. Phagocytosis assays with WT or knockdown (KD) THP-1 cells used IgG-opsonized sheep RBCs (ShRBCs) or IgG-opsonized streptavidin microbeads. Microscopy fields randomly selected and 200 macrophages counted (n=3, mean±s.e.m.). Images, engulfment of ShRBCs. Arrows denote phagocytic events. Scale bar: 10 µm. THP-1 cells with 13% CD47 levels had higher levels of engulfment than WT (⍰40%). *P<0.03. (B) SIRPα immunoprecipitation from lysates of THP-1 macrophages under basal conditions using anti-SIRPα (SE7C2 clone) and immunoblotted for phospho-tyrosine (pTyr; C-20 clone) (n=3, mean±s.e.m.). The normalized SIRPα pTyr signal increases linearly from zero in relation to the CD47 level. (C) (i) To quantify the effective affinity of CD47 for SIRPα in trans on WT and KD THP-1 macrophages, binding of fluorescent soluble CD47 was measured by flow cytometry and normalized to 8 µM data. All data fits y=A x/(Kd+x) (R2>0.93) for apparent dissociation constants (Kd). (ii) Kd increases linearly in relation to the CD47 level, and the non-zero intercept corresponds to the highest affinity for CD47–SIRPα as measured for human RBCs that lack SIRPα (Fig. 1B). Fig 2. PMID: 31964705

References

- Min C et al. Tim-4 functions as a scavenger receptor for phagocytosis of exogenous particles. *Cell Death Dis.* (2020)
- Hayes BH. et al. Macrophages show higher levels of engulfment after disruption of cis interactions between CD47 and the checkpoint receptor SIRPα. *Journal of Cell Science* (2020)
- Tsai, RK et al. Self inhibition of phagocytosis: the affinity of 'marker of self' CD47 for SIRPalpha dictates potency of inhibition but only at low expression levels. *Blood Cells, Molecules & Diseases* (2010)

Disclaimer

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