



# SZABO SCANDIC

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### SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

[mail@szabo-scandic.com](mailto:mail@szabo-scandic.com)

[www.szabo-scandic.com](http://www.szabo-scandic.com)

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

## Anti-Lipopolysaccharide [S69-4] Bulk Size Ab04122-23.0-BT

**Isotype and Format:** Rabbit IgG, Kappa

**Clone Number:** S69-4

**Alternative Name(s) of Target:** LPS; Kdo(2→8)[Kdo(2→4)]Kdo(2→4) Kdo; Kdo<sub>4</sub>; branched Kdo<sub>4</sub> tetrasaccharide; *Chlamydophila psittaci* Lipopolysaccharide tetrasaccharide; 3-deoxy-alpha-d-manno-oct-2-ulopyranosonic acid

**UniProt Accession Number of Target Protein:**

**Published Application(s):** SPR, TLC, ELISA, IF

**Published Species Reactivity:** *Chlamydophila psittaci*

**Immunogen:** The original antibody was generated by immunizing BALB/c mice with a synthetic neoglycoconjugate antigen — Kdo(2→8)[Kdo(2→4)]Kdo(2→4) Kdo-BSA (Kdo<sub>4</sub>-BSA).

**Specificity:** This antibody is specific for Kdo<sub>4</sub>, a *Chlamydophila psittaci* Lipopolysaccharide (LPS) tetrasaccharide (3-deoxy-alpha-d-manno-oct-2-ulopyranosonic acid (Kdo)) of the sequence Kdo(2→8)[Kdo(2→4)] Kdo(2→4)Kdo. This antibody specifically binds to the LPS of *Chlamydophila psittaci* but not to LPS from *Chlamydophila pneumoniae* or *Chlamydia trachomatis*. Furthermore, it binds exclusively to the LPS and O-deacylated LPS of *Chlamydophila psittaci*.

**Application Notes:** This antibody demonstrated high affinity and specificity for structures containing the branched Kdo<sub>4</sub> tetrasaccharide or the 2→4/2→4Kdo<sub>3</sub> trisaccharide, enabling differentiation of *Chlamydophila psittaci* from other chlamydial species. Furthermore, it shows potential for detecting individual molecular lipopolysaccharide (LPS) species and studying the enzymology of Kdo transferases. Its binding affinity to neoglycoconjugate antigens was assessed using ELISA checkerboard titrations. It displayed comparable affinity for 2→4/2→4Kdo<sub>3</sub>-BSA and Kdo<sub>4</sub>-BSA and weak binding was observed with 2→4Kdo<sub>2</sub>-BSA, 2→8Kdo<sub>2</sub>-BSA, and 2→8/2→4Kdo<sub>3</sub>-BSA, but only at high antibody and antigen concentrations. This antibody was also tested against BSA glycoconjugates containing 4'-monophosphoryl derivatives of deacylated *C. psittaci* LPS. It demonstrated similar reactivity with 2→4/2→4Kdo<sub>3</sub>-GlcNAc<sub>2</sub>-4P-BSA and Kdo<sub>4</sub>-GlcNAc<sub>2</sub>-4P-BSA. Further experiments confirmed its binding to isolated LPS and whole bacteria. Inhibition assays using free oligosaccharides as inhibitors in ELISA demonstrated that the antibody achieved 50% inhibition with 2→4/2→4Kdo<sub>3</sub>-allyl, Kdo<sub>4</sub>-allyl, and Kdo<sub>4</sub>-GlcNAc<sub>2</sub>-P<sub>2</sub> at concentrations of 4.4 μM, 1.7 μM, and 2.1 μM, respectively. An immunofluorescence (IF) assay on infected L929 and HL cells confirmed the antibody's recognition of a consistent epitope in *C. psittaci* inclusions, while remaining negative for *Chlamydophila pneumoniae* and *Chlamydia trachomatis*. Additional IF testing using various chlamydial strains confirmed the antibody's specificity, as all four tested *C. psittaci* strains displayed positive staining. Surface plasmon resonance (SPR) binding studies were conducted to determine the

interaction between the original antibody format (mouse IgG1) with various ligands; the dissociation constants ( $K_d$ ) for the immobilized antibody were measured for Kdo<sub>4</sub>-GlcNAc<sub>2</sub>-P<sub>2</sub>, 2→4/2→4Kdo<sub>3</sub>-GlcNAc<sub>2</sub>-P<sub>2</sub>, and 2→8/2→4Kdo<sub>3</sub>-GlcNAc<sub>2</sub>-P<sub>2</sub>, resulting in  $K_d$  values of 10 μM, 20 μM, and 200 μM, respectively. When the immobilized scFv version of the antibody was used, the binding constants at 150 mM NaCl were 10 μM, 100 μM, and 800 μM for Kdo<sub>4</sub>-GlcNAc<sub>2</sub>-P<sub>2</sub>, 2→4/2→4Kdo<sub>3</sub>-GlcNAc<sub>2</sub>-P<sub>2</sub>, and 2→8/2→4Kdo<sub>3</sub>-GlcNAc<sub>2</sub>-P<sub>2</sub>, respectively. The SPR studies revealed that this antibody exhibits a higher affinity for Kdo<sub>4</sub>-GlcNAc<sub>2</sub>-P<sub>2</sub> compared to 2→4/2→4Kdo<sub>3</sub>-GlcNAc<sub>2</sub>-P<sub>2</sub>, and significantly reduced affinity for the Kdo-trisaccharide containing 2→8-linked terminal Kdo (Müller-Loennies et al., 2006; PMID: 16282606).

**Antibody First Published in:** Müller-Loennies et al. A monoclonal antibody against a carbohydrate epitope in lipopolysaccharide differentiates *Chlamydophila psittaci* from *Chlamydophila pecorum*, *Chlamydophila pneumoniae*, and *Chlamydia trachomatis* *Glycobiology*. 2006 Mar;16(3):184-96. doi: 10.1093/glycob/cwj055 PMID:16282606

**Note on publication:** The original publication discusses the development of a monoclonal antibody that can differentiate *Chlamydophila psittaci* from other *Chlamydophila* species and *Chlamydia trachomatis* based on a specific carbohydrate epitope in the lipopolysaccharide.

## Product Form

**Size:** 1 mg Purified antibody in bulk size.

**Purification:** Protein A affinity purified

**Supplied In:** PBS only.

**Storage Recommendation:** Store at 4°C for up to 3 months. Note, this antibody is provided without added preservatives, it is therefore recommended this antibody be handled under sterile conditions. For longer storage, aliquot and store at -20°C.

**Concentration:** 1 mg/ml.

Important note - This product is for research use only. It is not intended for use in therapeutic or diagnostic procedures for humans or animals.