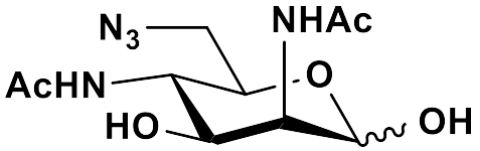
Langford Locks
Kidlington
Oxfordshire
OX5 1LH
Telephone: +44(0)1865 595230

Product Datasheet

Product Name: LegioTag

Product code: CLK83101-100

Product: 6-Azido-2,4-diacetamido-2,4,6-trideoxy-D-mannose



Structural formula of LegioTag

CAS Number: 1447950-85-7

Chemical Composition: $C_{10}H_{17}N_5O_5$

Concentration: 10mM

Form: Liquid, sterile dH_20

Volume: 100µl

Storage: Store at -20°C upon receipt or -80°C for long-term storage.

Description: 6-Azido-2,4-diacetamido-2,4,6-trideoxy-D-mannose ($Man_2NAc_4NAc_6N_3$) is an analogue of the natural 2,4-diacetamido-2,4,6-trideoxy-D-mannose that contains an azido moiety.

Application: Species-specific detection and labeling of *Legionella pneumophila*.
Strains tested include; *L. pneumophila* SG1 (Paris), *L. pneumophila* SG1 (Lens), *L. pneumophila* SG1 (Philadelphia), *L. pneumophila* SG3, *L. pneumophila* SG4, *L. pneumophila* SG5, *L. pneumophila* SG6.

Notes: LegioTag is an azide-functionalized Legionaminic acid (LEG) molecule for metabolic lipopolysaccharides (LPS) labeling of *Legionella pneumophila*. LEG is an essential lipopolysaccharide which is incorporated by *L. pneumophila* into its cell wall during active glycan biosynthesis. When cell-permeable LegioTag is added to an actively growing culture containing *L. pneumophila* it is intracellularly processed and utilized instead of its natural LEG counterpart. Using Cu(I)-catalyzed (CuAAC, terminal alkyne) or Cu(I)-free (SPAAC, strained alkyne) 'click chemistry' between an azide and an alkyne or cyclooctyne, this azide-modified glycan can then be labelled with either a fluorescent alkyne for imaging or a biotin alkyne for purification. Azide and alkyne groups conjugate to one another with high efficiency but do not react or interfere with other functional groups found in biological samples.

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Example Method: *L. pneumophila* culture in log growth phase is diluted in fresh Buffered Yeast Extract (BYE) broth (*e.g.* 1:100, final volume 0.2 ml) with addition of 20µl (1mM) LegioTag. Culture is then incubated overnight at the appropriate temperature. Following incubation, culture is washed with phosphate buffer prior to labeling by 'click chemistry'.

Materials Required but Not Provided:

1. Cell Reaction Buffer Kit *e.g.* Click-iT™ Cell Reaction Buffer Kit (Thermo Fisher Scientific).
2. Alkyne labelled probe *e.g.* Click-iT™ Alexa Fluor™ 488 sDIBO Alkyne *or* Click-iT™ Biotin sDIBO Alkyne (Thermo Fisher Scientific)

References:

1. Dumont et al. (2012). Click-mediated labeling of bacterial membranes through metabolic modification of the lipopolysaccharide inner core. Angew Chem Int Ed Engl. 51(13):3143-6.
2. Fugier et al. (2015). Rapid and Specific Enrichment of Culturable Gram Negative Bacteria Using Non-Lethal Copper-Free Click Chemistry Coupled with Magnetic Beads Separation. PLoS ONE 10(6): e0127700.
3. Mas Pons et al. (2014). Identification of living Legionella pneumophila using species-specific metabolic lipopolysaccharide labeling. Angew Chem Int Ed Engl. 53(5):1275-8.