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Product Datasheet

Product Name: BacTag

Product code: CLK83100-100

Product: Ammonium 8-azido-3,8-dideoxy-D-manno-octulosonate

CAS Number: 1380099-68-2

Chemical Composition: C₈H₁₆N₄O₇

Quantity: 10mM

Form: Liquid, sterile dH₂0

Volume: 100µl

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

Description: 8-Azido-3,8-dideoxy-D-manno-octulosonic acid (Kdo-N₃) is an analogue of the natural 3-deoxy-D-manno-octulosonic acid (Kdo) that contains an azido moiety.

Application: Detection and labeling of Gram-negative bacteria. Strains tested include; *Legionella pneumophila, Salmonella typhimurium, Escherichia coli O86, Escherichia coli K12*.

Notes: BacTag is an azide-functionalized 3-Deoxy-D-manno-octulosonic acid (KDO) molecule for metabolic lipopolysaccharide (LPS) labeling of Gram-negative bacteria. KDO is an essential component of Gram-negative bacteria cell membrane which is incorporated into the cell wall during active glycan biosynthesis. When cell-permeable BacTag is added to an actively growing culture containing Gram-negative bacteria it is intracellularly processed and utilized instead of its natural KDO 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.







N₃

Structural formula of BacTag



Example Method: Gram-negative bacteria culture in log growth phase is diluted in fresh media (*e.g.* 1:100, final volume 0.2 ml) with addition of 20µl (1mM) BacTag. 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.* <u>Click-iT[™] Cell Reaction Buffer Kit</u> (Thermo Fisher Scientific).
- Alkyne labelled probe *e.g.* <u>Click-iT[™] Alexa Fluor[™] 488 sDIBO Alkyne</u> or <u>Click-iT[™] Biotin sDIBO</u> <u>Alkyne</u> (Thermo Fisher Scientific)

References:

- 1. <u>Dumont et al. (2012).</u> Click-mediated labeling of bacterial membranes through metabolic modification of the lipopolysaccharide inner core. Angew Chem Int Ed Engl. 51(13):3143-6.
- 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. <u>Mas Pons et al. (2014).</u> Identification of living Legionella pneumophila using species-specific metabolic lipopolysaccharide labeling. Angew Chem Int Ed Engl. 53(5):1275-8.



