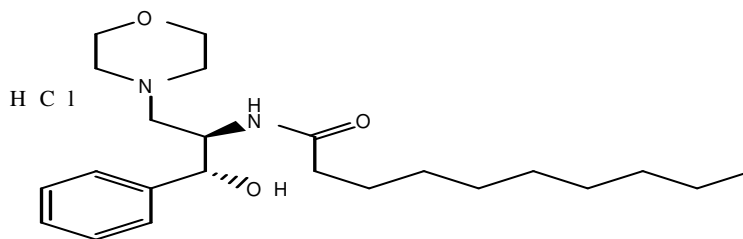# MATREYA NEWSLETTER

FOR GLYCO/SPHINGOLIPID RESEARCH
AUGUST 2014

Use of D-*threo*-PDMP In Atherosclerosis

Glycosphingolipids (GSL) are integrated components of cell membranes and they serve as messengers and growth factors.

Dr. Chatterjee and his coworkers at the Johns Hopkins University studying the effects of D-*threo*-PDMP on GSL transferase enzymes found that D-*threo*-PDMP not only inhibited the GSL synthesis but also ameliorated atherosclerosis. They measured i) aortic intima-media thickening and ii) vascular stiffness by aortic pulse-wave velocity.

D-*threo*-PDMP decreased cholesterol and triglyceride levels, by raising the expression of SREP2, low-density lipoprotein receptor, HMGCo-A reductase and cholesterol efflux genes (eg. ABCG5, ABCG8).

D-*threo*-PDMP affected very-low-density lipoprotein catabolism by increasing gene expression for lipoprotein lipase and very-low-density lipoprotein receptor. D-*threo*-PDMP decreased the total levels of cholesterol and lactosylceramide levels. Dr. Chatterjee found that D-*threo*-PDMP's inhibiting of GSL synthesis may be a novel approach to ameliorate atherosclerosis and arterial stiffness.

Matreya offers the following products for this research:

Catalog#	Product Description	Size	Purity
1756	D-*threo*-PDMP	10 mg	98+%
1500	Lactosylceramides	1 mg	98+%

References:
Chatterjee S, Bedja D, Mishra S, Amuzie C, Avolio A, Kass DA, Berkowitz D, Renehan M. Inhibition of glycosphingolipid synthesis ameliorates atherosclerosis and arterial stiffness in apolipoprotein e-/- mice and rabbits fed a high-fat and -cholesterol diet. Circulation 2014, 129(23):2403-2413

INSIDE THIS ISSUE

Ceramide Trihexoside Bottom-Spot as a Receptor to Detect Shiga Toxin 2 from STEC*

**Lingzi Xiaoli, Dept. of Food Science, Penn State University**
Kakolie Goswami, Dept. of Food Science, Penn State University
Dr. Edward George Dudley, Dept. of Food Science, Penn State University

Shiga toxin producing *Escherichia coli* (STEC) are notorious foodborne pathogens. Upon ingestion of contaminated food, the bacteria establish themselves in the colon and produce Shiga toxin (Stx) which translocates through the endothelial membrane and reaches the target cells (1–3). Disease symptoms first present as a mild diarrhea that can progress to bloody diarrhea and further lead to hemolytic uremic syndrome or hemorrhagic colitis. There are two immunologically distinct Shiga toxin, designated Shiga toxin 1 (Stx1) and 2 (Stx2). Studies have shown that Stx2 is more potent than Stx1 (4). While Stx can be detected by commercially available kits, these methods are not quantitative. Additionally, the only commercially available anti-Stx antibodies are mouse monoclonals, so designing an enzyme-linked immunosorbent assay (ELISA) requires one to generate antibodies from another species to use for capture or detection. We were interested in designing an ELISA using only commercially available components, and below we describe how we accomplished this using ceramide trihexoside (also known as globotriaosylceramide, Gb3, and CTH) obtained from Matreya.
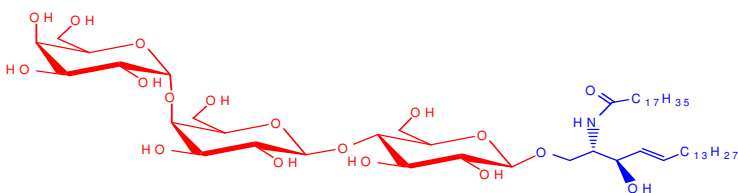
Catalog# 1513, Ceramide trihexosides top spot (non-hydroxy acyl)
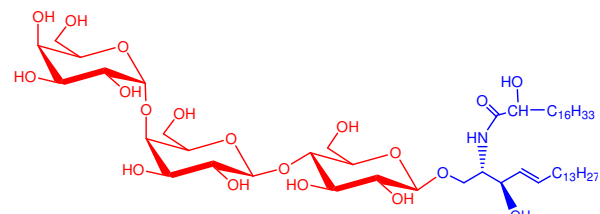
Catalog# 1514, Ceramide trihexosides bottom spot (hydroxy acyl)
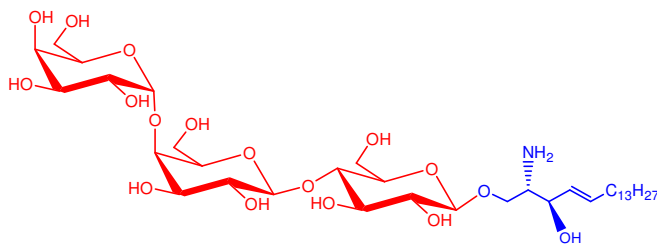
Catalog# 1520, *lyso*-Ceramide trihexoside

Shiga toxin 2 binds to the glycolipid globotriaosylceramide (Gb3) on the kidney epithelium. In our study, three different types of CTH were individually used to coat a polystyrene 96 well plate. Both the top (non-hydroxy acyl) and bottom (hydroxy acyl) spot of CTH as well as the semisynthetic *lyso*-CTH were compared, and we concluded that the bottom spot CTH was most sensitive for Stx quantification. We also investigated the efficacy of different solvents to dissolve the CTH bottom spot. Hot methanol (at 55°C), room temperature methanol and chloroform: methanol (2:1) were tested. CTH bottom spot dissolved in the chloroform:methanol (2:1) gave the highest signal. We also tested a combination of cholesterol, lecithin and CTH (bottom spot), however the bottom spot alone showed highest

Ceramide Trihexoside Bottom-Spot as a Receptor to Detect Shiga Toxin 2 from STEC*

The method involves dissolving the CTH bottom spot in chloroform:methanol (2:1). We prepared a working solution by mixing the dissolved CTH in methanol to a final concentration of 25 µg/mL. A coating of 100µL was pipetted onto the wells of the microtiter plate and allowed to evaporate in a fume-hood with constant air flow. Once the evaporation was complete, the uncoated sites in the well were blocked with blocking buffer (0.01M phosphate buffer saline, 4% BSA and 0.05% Tween-20) and incubated at 4°C overnight. The next day, the wells were washed with PBS containing 0.05% Tween-20 (PBS-T), and 100µL of samples containing the Stx2 were added. Following a 1 hr incubation, the plates were washed with PBS-T to remove unbound Stx2 and mouse anti-Stx2 primary antibody was added to a final concentration of 0.1 µg/well. The primary antibody binds to the Stx2, which in turn is bound to the CTH receptor. After a 1 hour incubation, the wells were washed again and secondary antibody was added. The secondary antibody was goat anti-mouse antibody, which has a horse-radish peroxidase (HRP) conjugate. After a 1 hr incubation and washes, the substrate TMB (3,3′,5,5′-tetramethylbenzidine) was added. The HRP from the secondary antibody breaks down TMB giving a blue color. This reaction is stopped by adding 2M H_2SO_4. After addition of the stop solution, a yellow color develops and the intensity of this is quantified by a plate reader. The intensity of the color corresponds to the amount of Stx2 present in the sample. Standards were run with each reaction that allows the exact quantification of the Stx2.
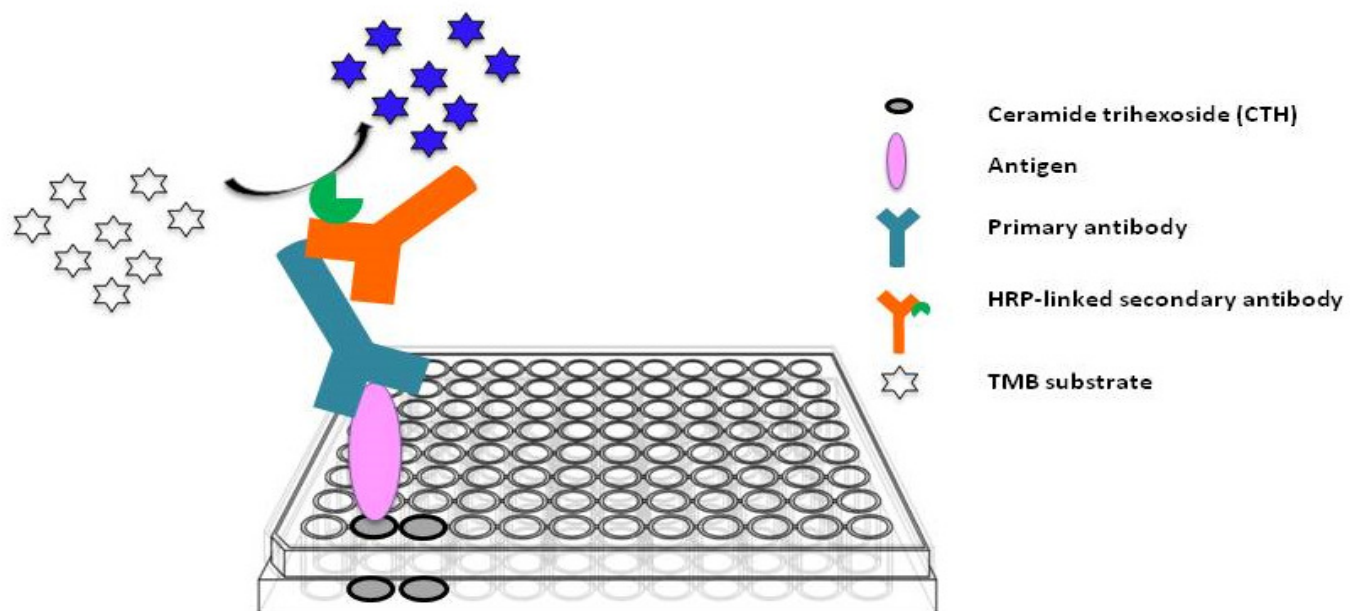

Fig.1 Application of CTH in sandwich ELISA

Our R-ELISA (Receptor ELISA) can successfully detect and quantify Stx2, and routinely a linear curve is obtained between 20 and 350 ng/mL Stx2 (Figure 2). This method can also be used to detect Stx1 by using different primary antibodies. Unlike many more expensive commercially available kits for detecting Stx, our method can distinguish Stx2 from Stx1. It also allows multiple samples to be processed at the same time, and all necessary components for performing this assay are commercially available.

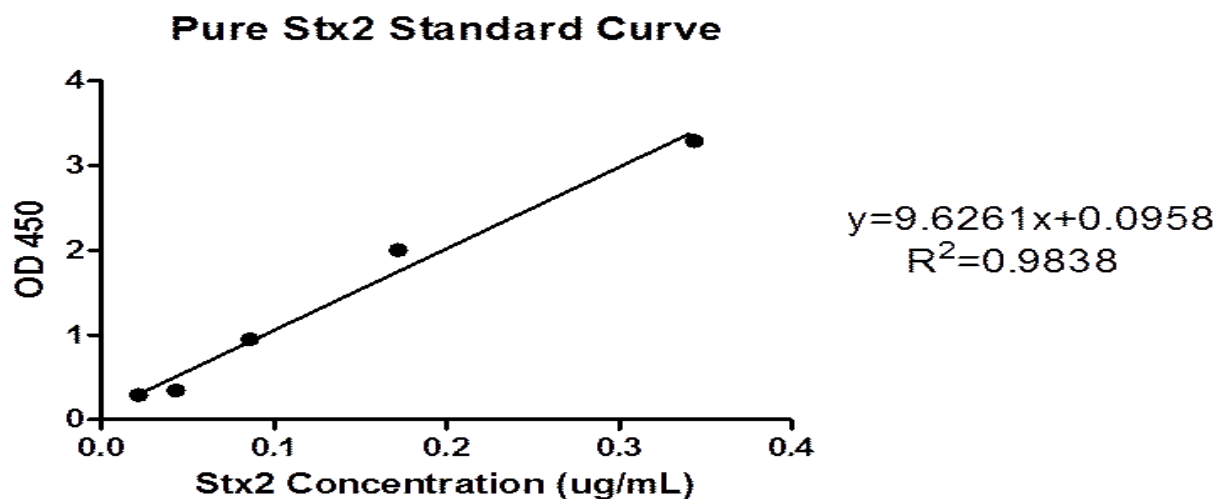# Ceramide Trihexoside Bottom-Spot as a Receptor to Detect Shiga Toxin 2 from STEC*

$$y = 9.6261x + 0.0958$$
$$R^2 = 0.9838$$

Fig. 2 Linear curve of pure Stx2 sample by using R-ELISA

Glycosphingolipids used in this article:

Catalog#	Product Description	Size	Purity
1513	Ceramide trihexosides (top spot)	0.5 mg	98+%
1514	Ceramide trihexosides (bottom spot)	0.5 mg	98+%
1520	*lyso*-Ceramide trihexoside	1 mg	98+%

Other sphingolipids, including an extensive list of well defined ceramide trihexosides (globotriaosylceramides), are available at www.matreya.com.

References:

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4. **Fuller C a, Pellino C a, Flagler MJ, Strasser JE, Weiss A a**. 2011. Shiga toxin subtypes display dramatic differences in potency. Infect. Immun. **79**:1329–1337.

5. **Gallegos KM, Conrady DG, Karve SS, Gunasekera TS, Herr AB, Weiss A a**. 2012. Shiga toxin binding to glycolipids and glycans. PLoS One **7**:e30368.