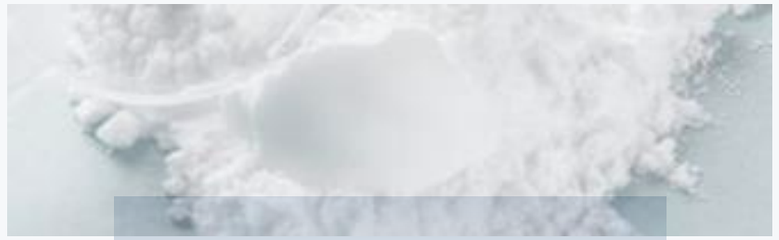
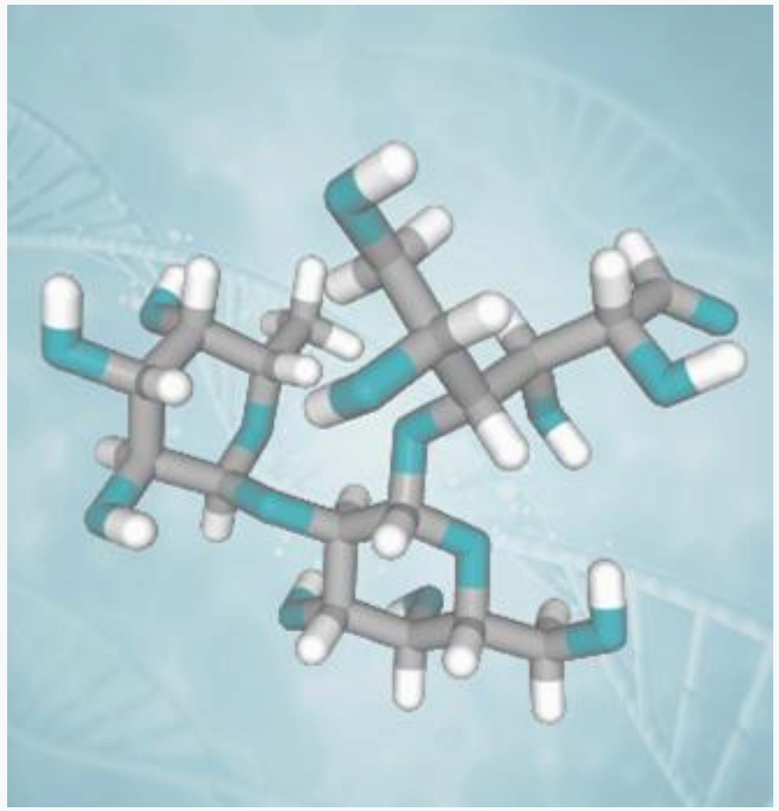
Private and Strictly Confidential





Advanced Protein Technologies Corp.

AP Technologies 2’-FL

Cell factory for human wellbeing.

2020.09

Table of Content

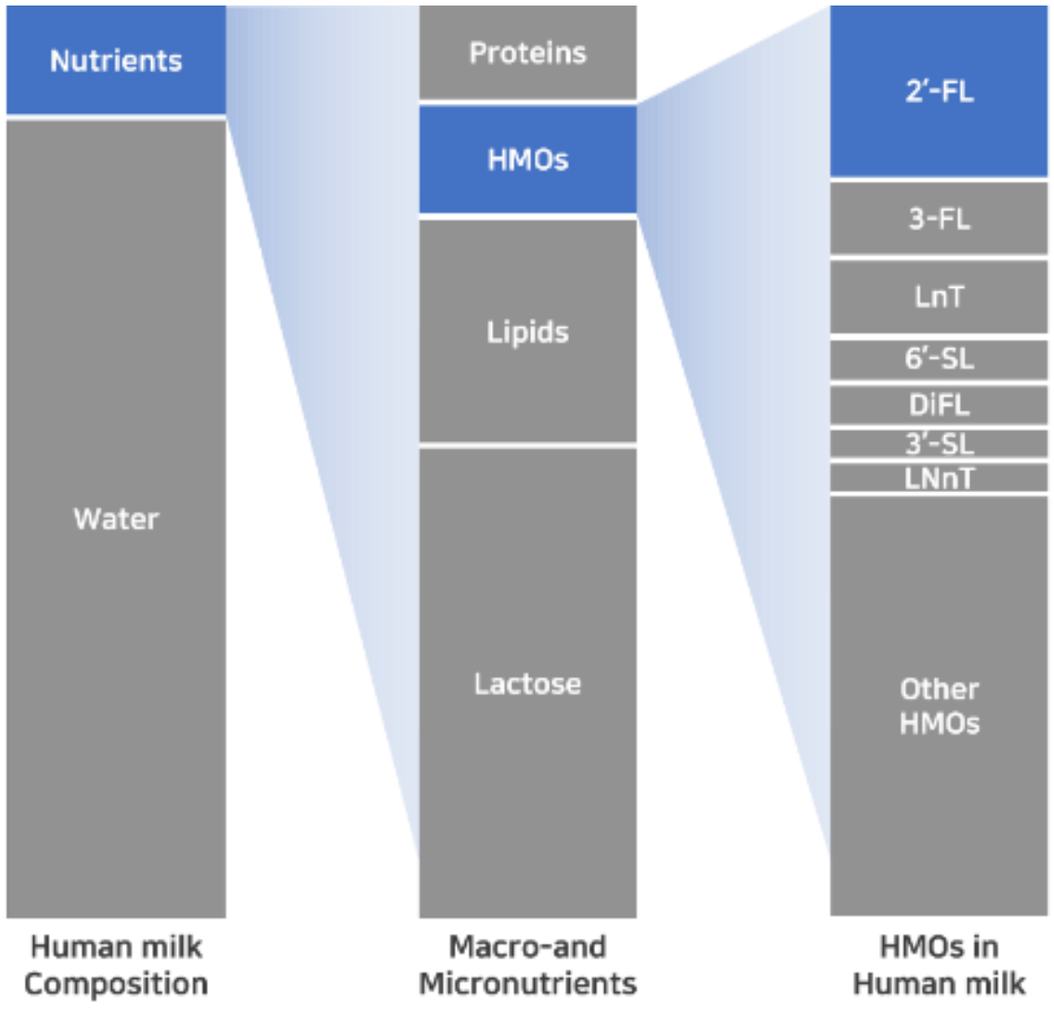
AP Technologies 2’-FL

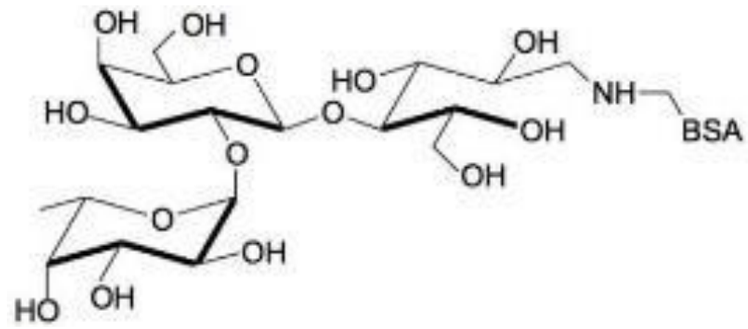
Cell factory for human wellbeing.

2’-Fucosyllactose (2’-FL)

HMOs make up 8~15g per liter of breast milk.

2’-FL takes up the largest percentage (approximately 2.7g per liter)





Oligosaccharide	Mean concentration, g/L of breast milk
2’-FL	2.7(1.88~4.9)

2'-Fucosyllactose is a three-sugar compound made up of lactose and L-fucose and accounts for about 30% of Human Milk Oligosaccharides (HMOs) in breast milk.

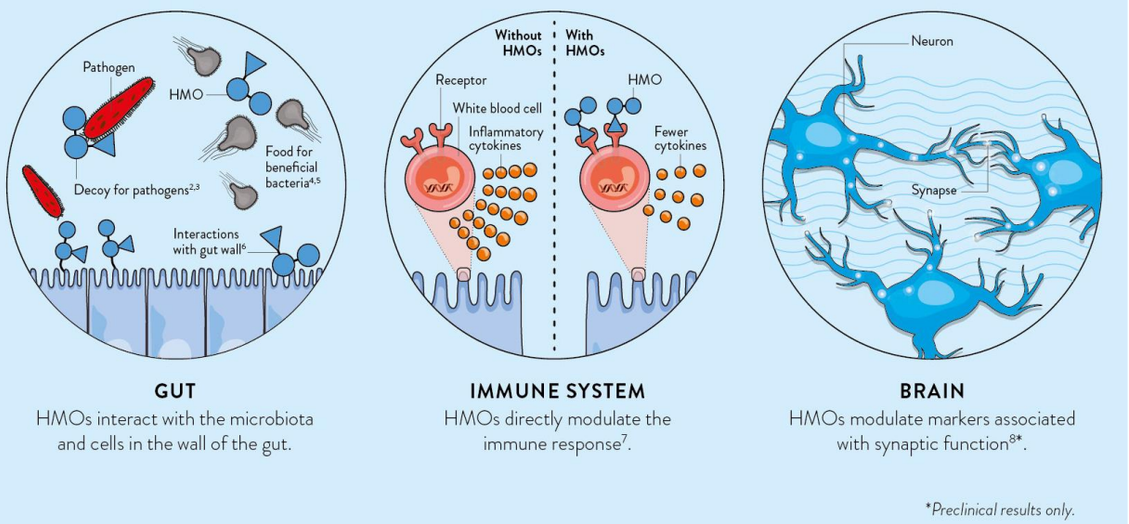
2’-FL’s Functions

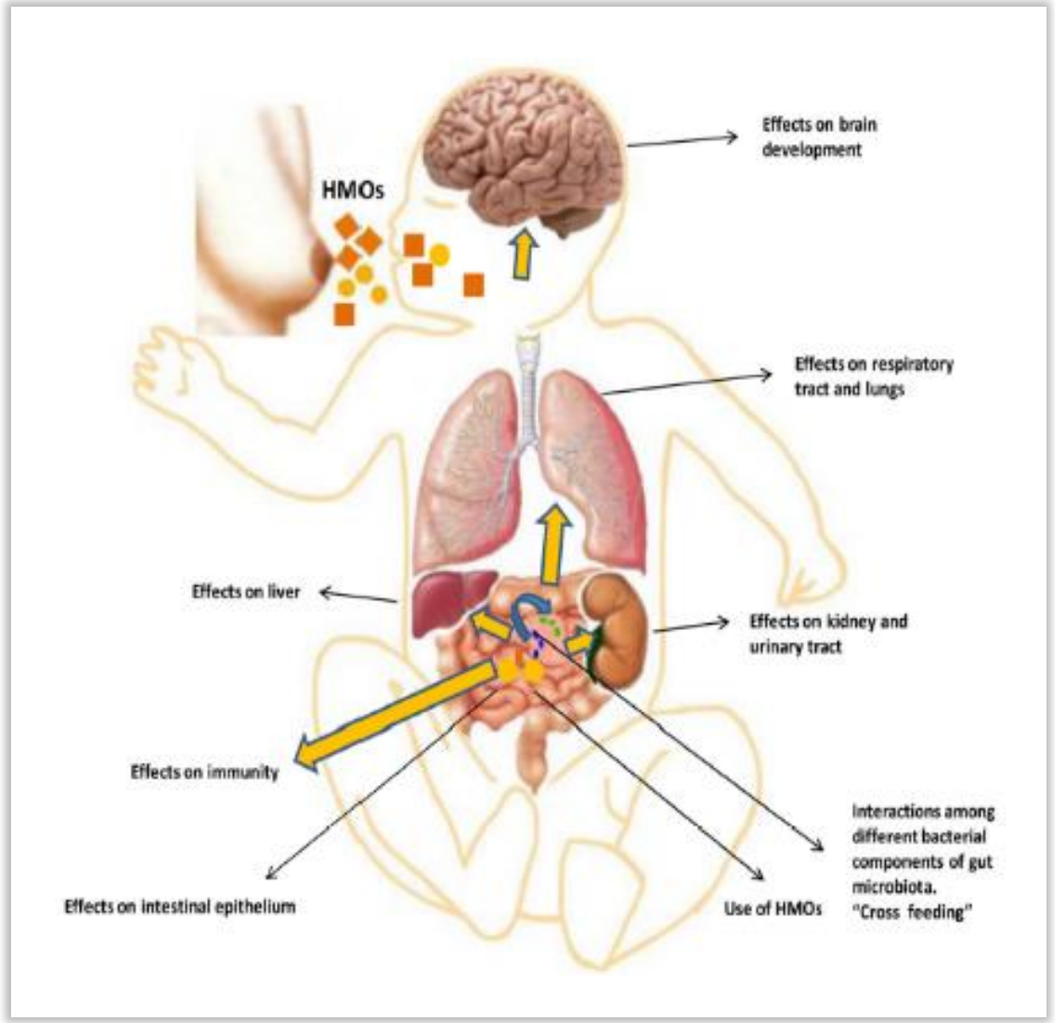
2’-FL Aids Formation of Healthy Microbiome, Immune Activation & Brain Development

HMO Functions

- HMOs play a crucial role in the formation of a healthy microbiome
  - Encourage the growth of beneficial microorganisms
  - Prevent the growth of harmful bacteria and viral infections
- Immune Activation and reduce Inflammation
- Growth & Protection of Brain Cells

Main Physiological Activity of 2’-FL





Source : Fanos V.(2018) J Pediatr Neonat Individual Med. 7(1):e070137

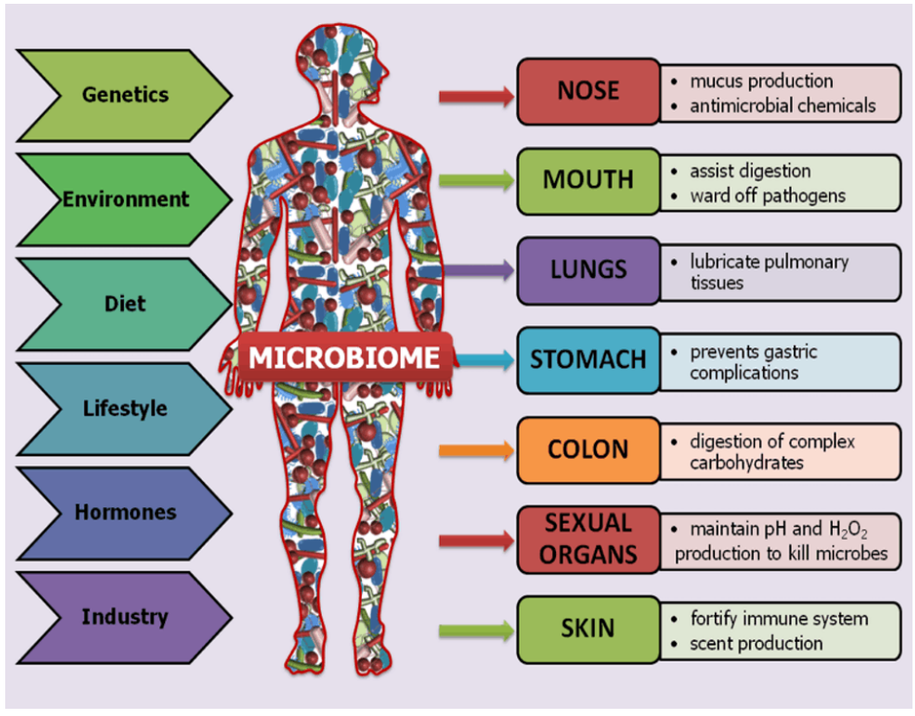
2’-FL’s functions include useful microbial growth, immunosuppression, anti-inflammatory effects, brain development, etc.

2’-FL’s Importance in Microbiome

- **2’-FL’s selective Bifidus Factor/ only feeds targeted probiotics**
- **Priority is given to *Bifidobacteria* in the formation of intestinal microbiome → Immune Activation & Organ Formation**

Human Microbiome

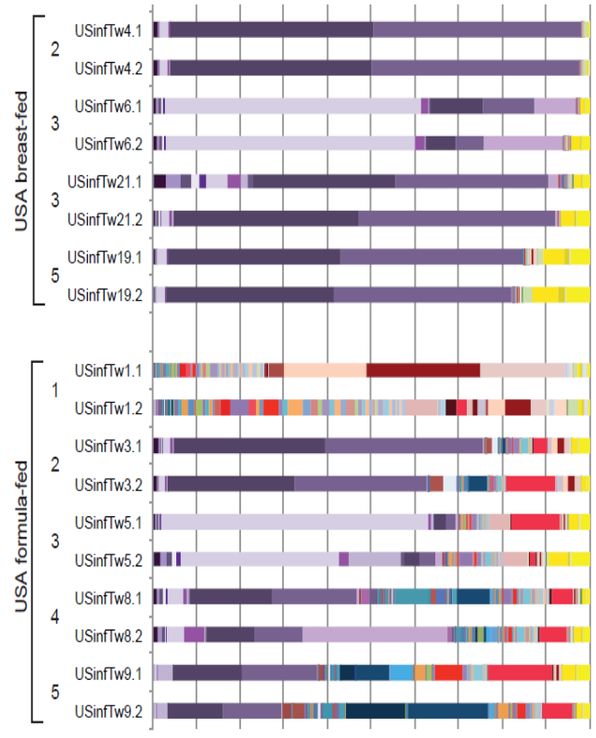
- Interaction between Environment & Human Microbiome



Human Microbes : The power within (2018)

Intestinal Flora Breastfed vs Infant Formula-fed Infants

- Breastfed Infants
  - Mostly *Bifidobacterium* found in intestines
- Formula-fed Infants
  - Various microorganisms found in intestines (*Bifidobacterium* relatively low)



Purple shade: Bifidobacterium species
Blue shade: Bacteroides species
Red shade: Species within Fimicutes
Yellow shade: Species within Proteobacteria

Purple bar indicates beneficial Bifidobacteria (beneficial probiotic)

***Bifidobacterium* Major Flora Formation → Immune Activation**

2’-FL Supporting Immune Development

- 2’-FL helps to increase immunity by increasing T Cells
- 2’-FL decreases inflammations shown by a decrease in B Cells

Populations in MLN (%)	REF	2’-FL
B cells (CD45RA+)	30.53 ± 2.54	18.13 ± 2.19*
T cells (Th and Tc cells)	62.01 ± 2.12	74.97 ± 2.03*
Th cells (CD4+ TCRαβ+ NK–)	46.21 ± 1.32	55.56 ± 1.45*
CD62L+	23.46 ± 3.10	29.33 ± 1.80*
CD25+	2.15 ± 0.53	2.64 ± 0.08
Tc cells (CD8+ TCRαβ+ NK- and TCRγδ+)	15.80 ± 0.81	19.41 ± 0.62*
TCRαβ+ (CD8+ TCRαβ+ NK–)	13.65 ± 0.81	16.91 ± 0.60*
TCRγδ+	2.15 ± 0.04	2.50 ± 0.05*
CD8+	1.52 ± 0.02	1.84 ± 0.06*
CD8–	0.62 ± 0.05	0.66 ± 0.05
NKT cells (TCRαβ+ NK+)	2.99 ± 0.46	3.08 ± 0.22
CD8+	1.72 ± 0.27	1.84 ± 0.11
CD8–	1.27 ± 0.19	1.24 ± 0.14
NK cells (TCRαβ- NK+)	3.43 ± 0.62	2.75 ± 0.16
CD8+	0.47 ± 0.11	0.50 ± 0.05
CD8–	2.96 ± 0.51	2.25 ± 0.13

Results are expressed as mean ± S.E.M. (n = 4/group).
**p < 0.05 compared to REF (by Student T-test).*
Th, T-helper; Tc, T-cytotoxic; NK, Natural Killer; NKT, Natural Killer T; REF, reference; 2′-FL, 2′-fucosyllactose.

Immunomodulatory and Prebiotic Effects of 2'-Fucosyllactose in Suckling Rats (2019)

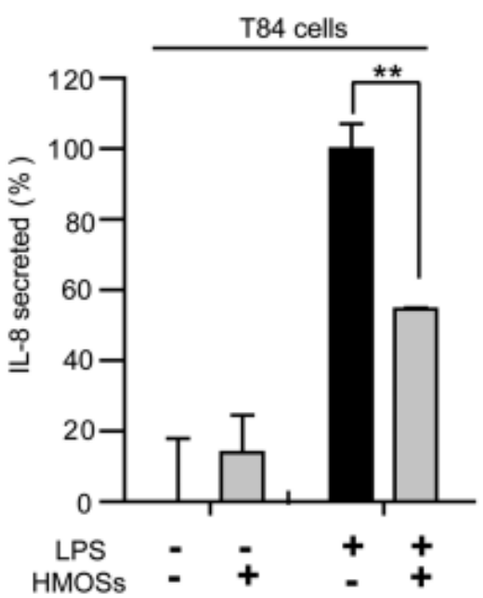
B cells (a type of white blood cell) increase when there are infections present in the body. A <u>decrease</u> in comparison with the control <u>indicates a lack of inflammation</u> in the body.

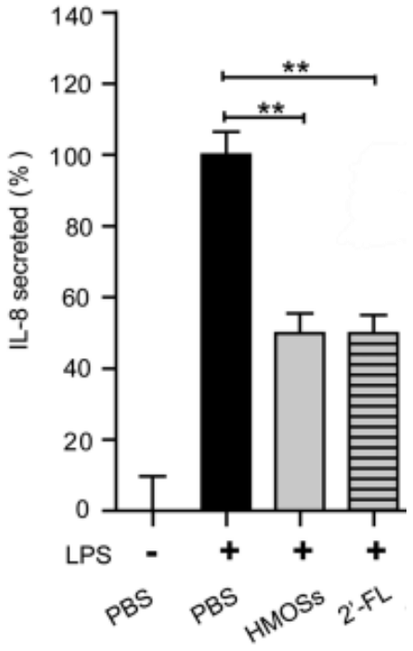
T cells help in immune response. An <u>increase</u> in comparison with the control indicates an <u>improved immune response</u>.

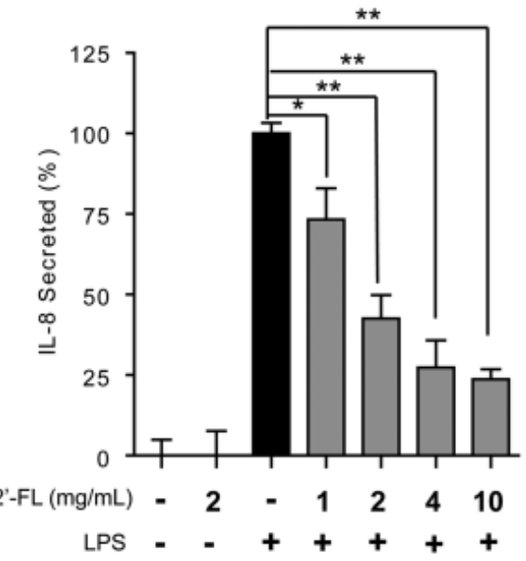
***2’-FL*→ Immune Activation**

2’-FL Reduces Inflammation

- **LPS-induced IL-8 secretion (inflammation) was inhibited by 2’-FL**
- **Higher doses of 2’-FL reduces the level of IL-8 secretion (inflammation)**







Abbreviation	Full Name	concentrations of milk oligosaccharides in literature [3, 40, 41] (mg/mL)	concentrations of milk oligosaccharides used (mg/mL)	inhibitory activity (%/mg)
HMOSs	Human milk oligosaccharides	5 -15	5	10.0 ±1.1
2 '-FL	2'-Fucosyllactose	2.5 ±1.2	2	25.0 ±1.0

The human milk oligosaccharide 2'-FL modulates CD14 expression in human enterocytes, thereby attenuating LPS-induced inflammation (2014)

Legend
LPS: Inflammation Stimulation
IL-8 (Interleukin-8): inflammation level

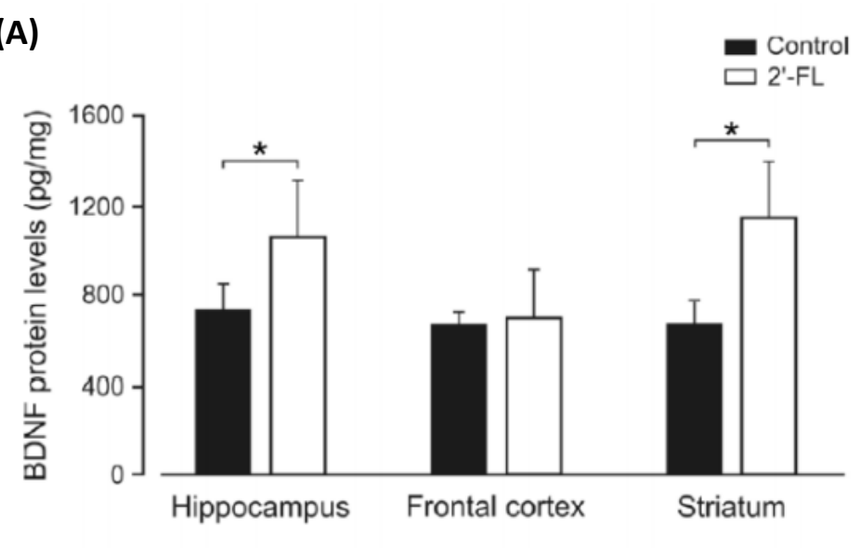
IL-8 (inflammation level) decreases in relation to increase in 2’-FL dose.

Inflammation levels controlled when treated with 2’-FL

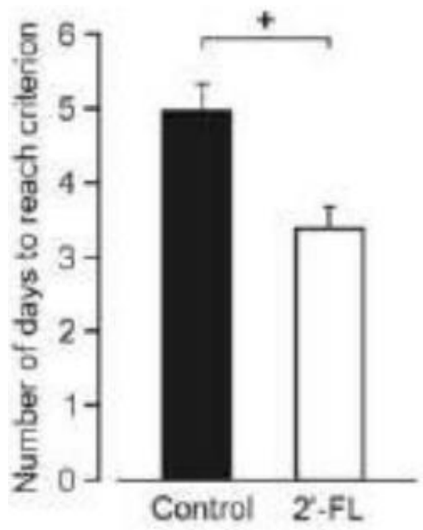
2’-FL Supports Cognitive Development

- **Increase in BDNF in both the hippocampus and striatum showed**

(A)



(B)



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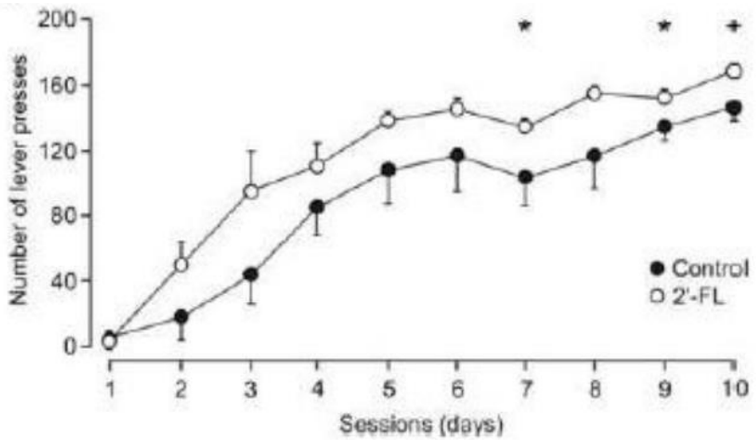


Effects of a 2'-FL on hippocampal long-term potentiation and learning capabilities in rodents

Legend
BDNF (Brain-derived neurotrophic factor): Indication of brain development

(A) Level of BDNF in the cortex, striatum and hippocampus of 2’-FL fed rats as compared to control. Both the hippocampus and striatum from 2’-FL fed rats showed increased expression of BDNF.

(B) 2’-FL-treated (6 weeks) rats reached the criterion in fewer sessions (*, P = 0.053) than the control group. The selected criterion was to press the lever up to 40 times/session and to repeat the same rate during the following session.

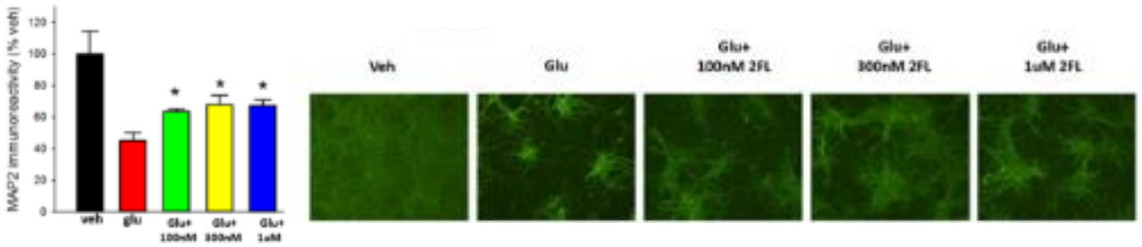
(C) 2’-FL rats performed more lever presses than their littermate controls (*, P ≤ 0.05)

2’-FL helps in Brain Development

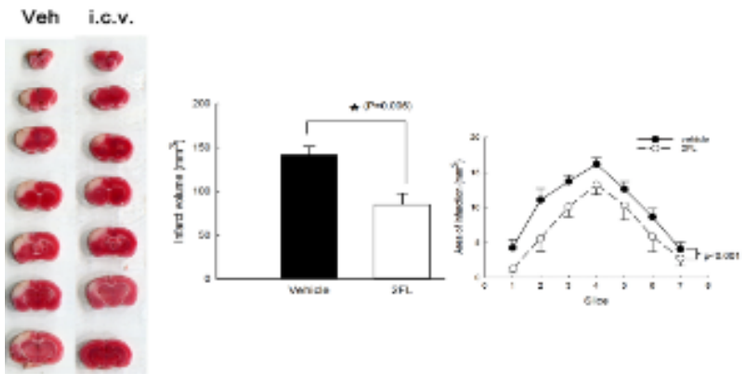
2’-FL Supports Brain Health

Neuroprotective action against Stroke (injection)

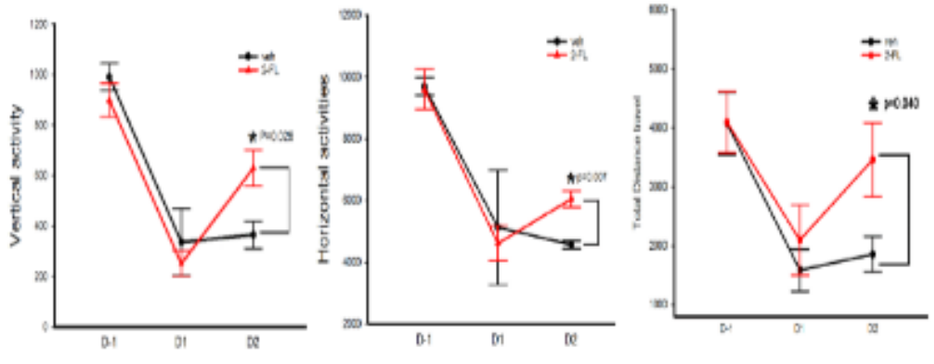
- 2’-FL prevents damage to MAP2 caused by glutamate even at low concentrations (less than 300nM)



- 2’-FL injection 30*μ*g/kg on stroke induced rats suppressed cerebral infraction formation

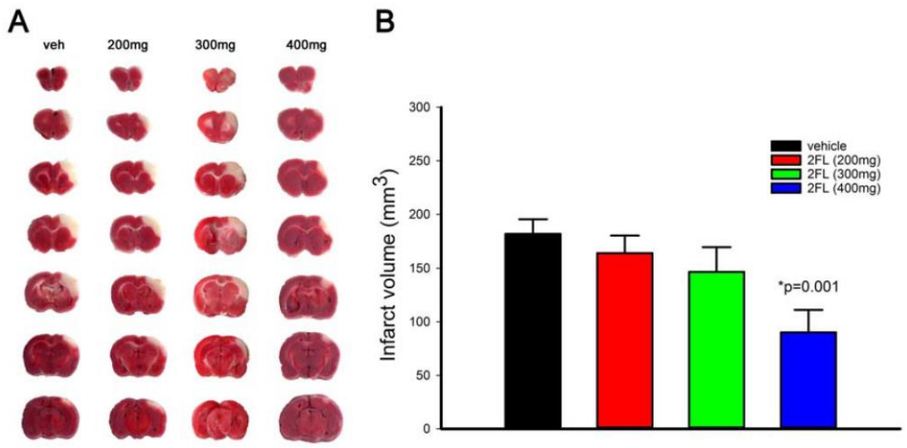


- Stroke induced rats’s locomotor behavior when injected with 2’-FL (30*μ*g/kg)

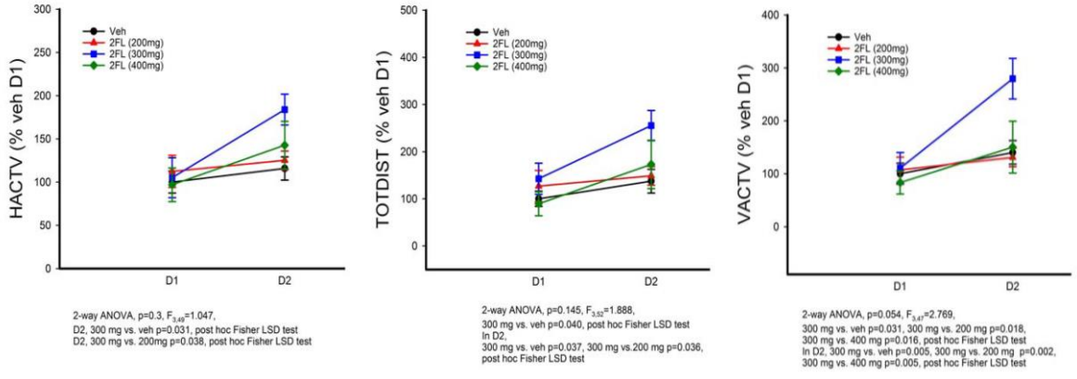


Neuroprotection against Stroke (Oral Administration)

- Identification of Brain Damage Prevention Effects Caused by Major Cerebral Arterial Stenosis
- Group that was administered with 2’-FL had a smaller area of brain injury



- Behavioural Analysis of Brain Damage Caused by Major Cerebral Arterial Stenosis
  - Significant difference in amount of activity between control group and group administered with 2’-FL



Human Milk Oligosaccharide 2’-Fucosyllactose Reduces Neurodegeneration in Stroke Brain (2019)

2’-FL Applications

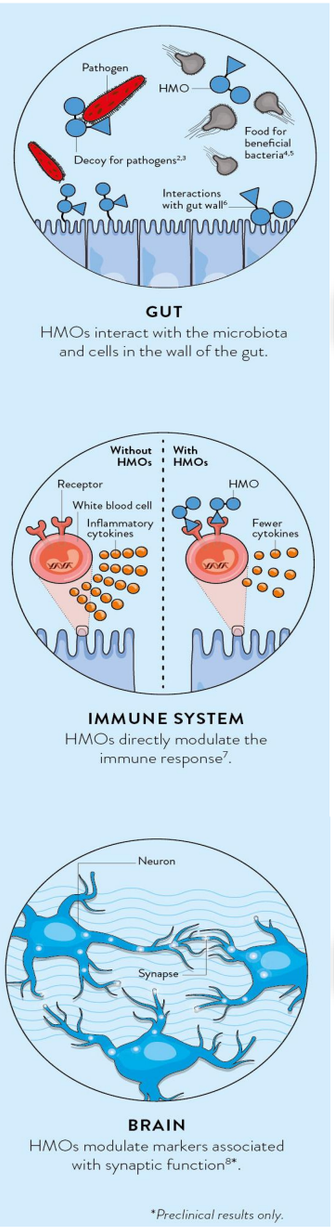
- **The Benefits of Breast Milk can be brought into Formula Milk, Functional Food and Cosmetics**
- **Possibility to Prevent and Treat Brain Health Related Diseases and Infections via the Microbiome**

✓ Foods

- Infant formula, Baby food
- Maternal Nutrition
- Prebiotics
- Synbiotics

✓ Household Products

- Oral care(Mouth Wash)
- Feminine hygiene
- Eye drop



✓ Cosmetics

- (Baby) Skincare
- Anti-aging, Fine dust care
- Skin microbiome control

✓ Pharmaceuticals

- Intestinal health care, CDI …
- Stroke & Neuroprotection
- Allergy, Atopy, Rheumatism

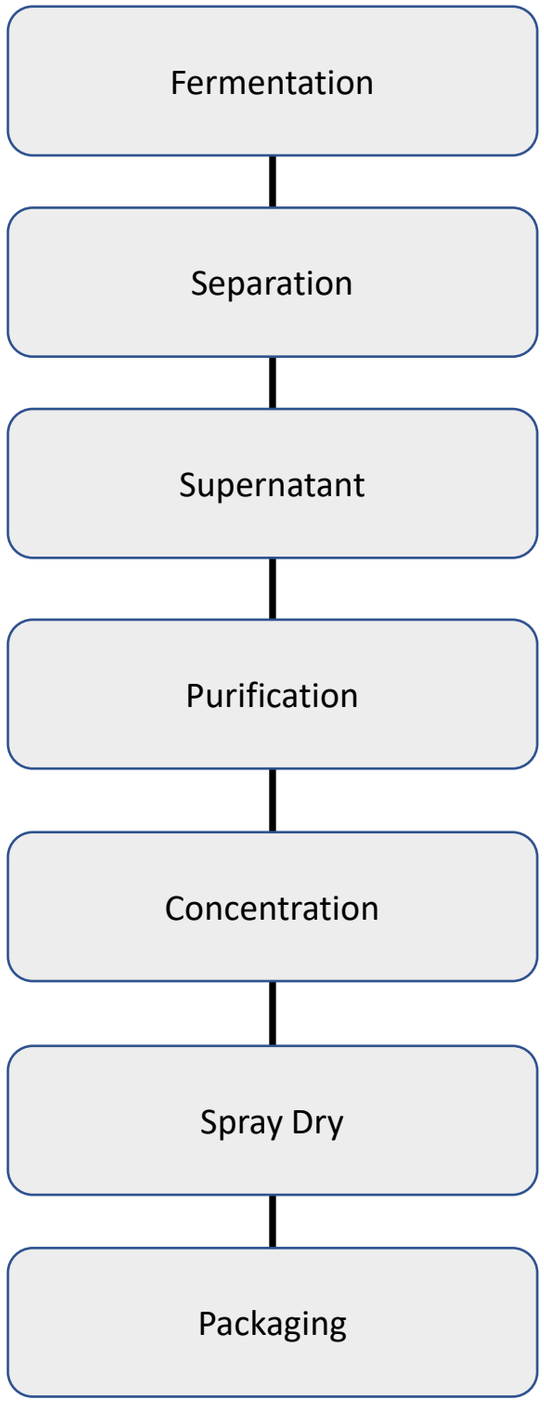
2’-FL Application Dosages

Proposed Food Uses	RACC	Proposed Level of Use (g/RACC)	Proposed Maximum L evel of Use
Infant formula (0 to 6 months), including ready-to-drink formula or formula prepared from powder	100 g[b]	0.24	2.4 g/kg(4.0 g/kcal)
Follow-on formula (6 – 12 months), including ready-to-drink formula or formula prepared from powder	100 g[b]	0.24	2.4 g/kg(4.0 g/kcal)
Growing-up(toddler) milks (12 – 36 months)	100 g[b]	0.24	2.4 g/kg
Infant meal replacement products such as PediaSure®	100 g[b]	0.24	2.4 g/kg(4.0 g/kcal)
Yogurt and juice beverages identified as “baby” drinks	120 g	1.2	10 g/kg
Other baby foods for infants and young children	7 to 170 g	0.084 to 2.04	12 g/kg
Formula intended for pregnant women (Pregnancy formulas, -9 to 0 months)	200 ml[b]	1.2	6.0 g/l
Yogurt	225 g	1.2	5.3 g/kg
Flavored milks, including chocolate milk, coffee drinks, cocoa, smoothies (dairy and fruit-based), other fruit and dairy combinations, yog urt drinks, and fermented milk drinks including kefir	240 ml	0.28	1.2 g/l
Ready to eat breakfast cereals for adults and children	15 g (puffed), 40 g (high-fiber), 60 g (biscuit-types)	1.2	80 g/kg(puffed) 30 g/kg(high-fiber) 20 g/kg(biscuit-types)
Fruit juices and nectars	240 ml	0.28	1.2 g/l
Desserts including fruit desserts, cobblers, yogurt/fruit combinations(“jun ior type” desserts)	110 g	1.2	10.9 g/kg
Sports, isotonic and energy drinks	240 ml	0.28	1.2 g/l
Meal replacement drinks for weight reduction	240 ml	1.2	5 g/l
Oral nutritional supplements and enteral tube feeding (11 years and older)	200 g[b]	4.0	20 g/kg
Cosmetics	-	0.5-1%	-

APT 2’-FL Manufacturing Process

Development of 2’-FL Production Strain at Biosafety Level 1 (BSL1)

2’-FL Manufacturing Process



- Transporter and Enzyme using BSL1 Safe Microorganisms
  * BSL1 (Bio-Safety Level 1) : Completely Safe Microorganism

- Feb 2019 Korean MFDS Strain Safety Application Completed

APT 2’-FL Cost Competitiveness & IP

Manufacturing Technological Competitiveness and Differentiation

Differentiated Production Strain

- APT’s 2’-FL GRAS* Production Strain Differentiation
  - Usage of *C. glutamicum* as a food production strain
  - Existing use for mass production of food ingredients minimising safety concerns

- Competitor Companies Overseas using *E. coli* for Production
  - Safety & Patent Concerns using the same *E. coli* strain

*GRAS : Generally Recognized As Safe

2’-FL Development Status

Advanced Protein Technologies Corporation
- GRAS microorganism *C. glutamicum* Registration and Obtaining of Patent
- Fermentation and Purification Technology Completed

Jennewein Biotechnologie GmbH
- Using *E. coli* as production strain
- 2015 US FDA & 2017 EU Registration
- 2018 Lawsuit with Glycosyn regarding production strain

GLYCOM
- Using *E. coli* as production strain
- 2015 US FDA & EU Registration
- 2′-FL Added to Nestle Infant Formula

Patent Status

- Patent (Reg No. 10-1731263)
  – 2’-FL Production Method using *C. glutamicum* (US, EU, China, Japan, Australia, Canada, India Application)
  – Related Patent: Cosmetic Products (Reg No. 10—1972925)

- Patent Application : Strain and Production Related Patent Strengthening and Applications
  – 2’-FL BSL1 Conversion Enzyme & Production Strain *C. glutamicum* Production Method
  – Application of 2’-FL in Stroke Treatment, Eye Drops
- Trademark : “Momstamin” (Korea, US, Europe, China, Japan)


특허증
CERTIFICATE OF PATENT

특허 Patent Number 제 10-1731263 호

출원번호 Application Number 제 10-2016-0050219 호

출원일 Filing Date 2016년 04월 25일

등록일 Registration Date 2017년 04월 24일

발명의 명칭 Title of the Invention
코리네박테리움 글루타미쿰을 이용한 2′-푸코실락토오스의 생산방법

특허권자 Patentee
서울대학교 산학협력단(114371-*******)
서울특별시 관악구 관악로 1 (신림동)

발명자 Inventor
등록사항란에 기재

위의 발명은 「특허법」에 따라 특허등록원부에 등록되었음을 증명합니다.
This is to certify that, in accordance with the Patent Act, a patent for the invention has been registered at the Korean Intellectual Property Office.

2017년 04월 24일

특허청
Korean Intellectual Property Office

특허청장
COMMISSIONER,
KOREAN INTELLECTUAL PROPERTY OFFICE

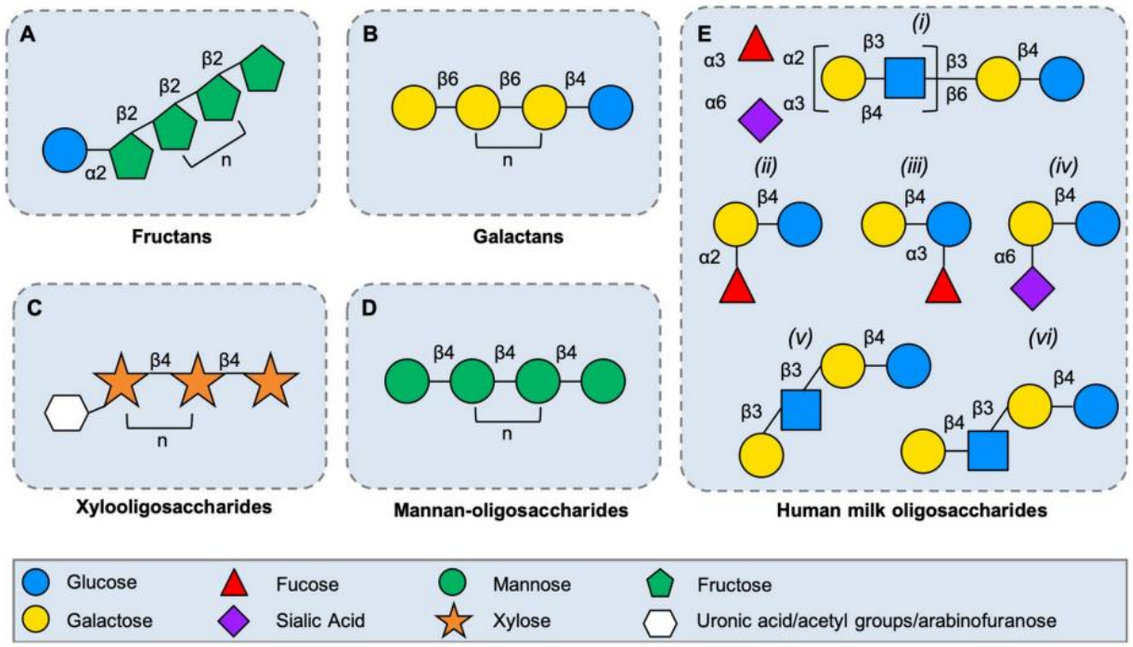
Momstamin

曼生泰
Momstamin

Oligosaccharide Comparison

- **HMOs are more structurally diverse hence creating specificity for Bifidobacteria and immune system**

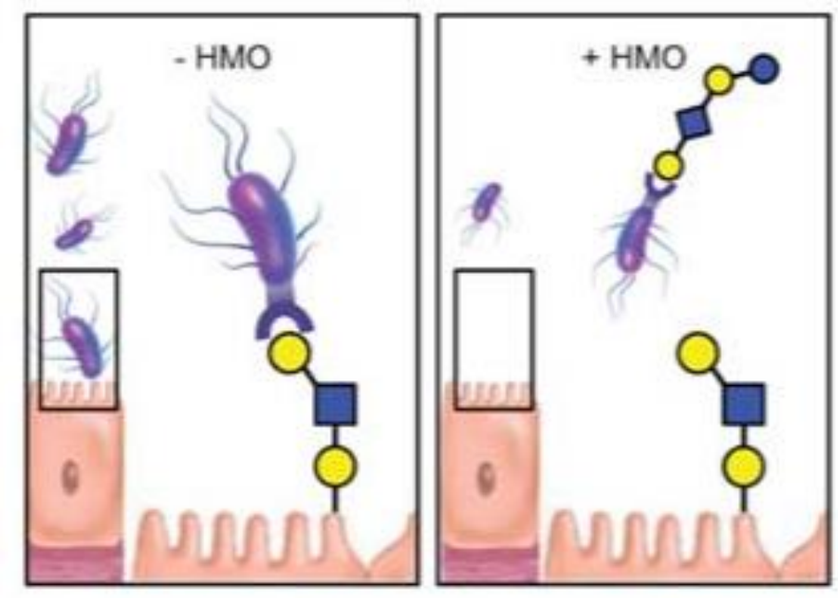
Physical Differences



Prebiotics: tools to manipulate the gut microbiome and metabolome (2019)

Comparison Chart

Oligosaccharide	Bifidus factor	Use by Non-bifido Bacteria	Exist in Breast Milk	Minimal dosage (g/day)
FOS	Y	Y	N	3
Lactosucrose	Y	Y	N	2
GOS	Y	Y	N	2.5
Lactulose	Y	Y	N	3
IMOS	Y	Y	N	10
XOS	N	Y	N	0.4~0.7
SBOS	Y	Y	N	5
Raffinose	Y	Y	N	3
2'-FL	Y	N	Y	1.87



Human Milk Oligosaccharides: Every baby needs a sugar mama (2012)

2'-FL is specific to Bifidobacteria

APT 2’-FL Details

Specifications

Physical Properties	White to off white/ ivory powder
Purity	≥94%
Water content	≤9.0%
Solubility in Water	500 g/L (25℃)
Heat Stability	Up to 250℃
Microbiology analysis	
Standard Plate Count, cfu/g	≤ 500
Yeast and Mold, cfu/g	≤ 100
Coliform, cfu/g	≤ 10
E. coli	Absent in 25 g
Cronobacter	Absent in 60 g
Staphylococcus aureus	Absent in 1 g
Salmonella	absent in 25 g
Endotoxins, EU/g	≤ 100

Product Details

HS Code	2940 0000
Sweetness	Low
Shelf Life	24 months
Storage Temperature	15-25℃
Packaging Size	1kg , 15 kg.