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MG
Metallogenics

For monitoring lithium concentration in Blood

Lithium Assay LS

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MG
Metallogenics

Assay Kit for Total Antioxidant Capacity and Thiol

Oxidative Stress Assay Kit (TAC / Thiol)

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BioVerde

Cryopreservation media for ES/iPS cells by Vitrification

DMSO/Serum/Protein/Xeno-free StemCell Keep

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2015

World's standard α – Galactosyl Ceramide

KRN7000 α – Galactosyl Ceramide

Lithium Assay LS

For more information : http://www.funakoshi.co.jp/exports_contents/46145

Lithium in Serum / Plasma can be measured by microplate reader!

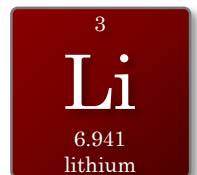
Background

Lithium is known to be related to the stimulation or suppression of the release of Serotonin, Dopamine, and Adrenaline from neuronal cell. This causes 1) increase numbers of White Blood Cell, 2) lowering blood-pressure, and 3) effect for reproduction.

In medication, lithium carbonate are being used for treatment of bipolar disorder as the lithium donor. However, lithium carbonate is absorbed by a gastrointestinal and overdose of a lithium may cause nephropathy and other critical side effects. Therefore, supervised administration of lithium carbonate is required and important. Serum lithium levels higher than 1.5 mM indicate a significant risk of intoxication. Recently, lithium effects for the oxidative stress-induced diseases has been reported. Therefore, researches about the effect of lithium based drugs against other diseases are reconsidered.

Features

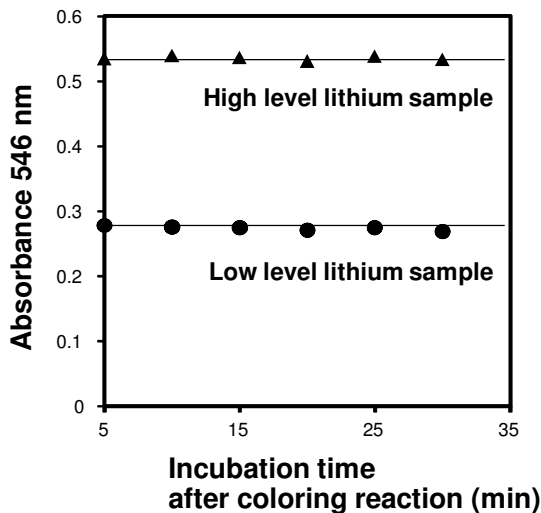
- Using conventional microplate reader for the assay. No gas barrier equipment is required.
- High throughput measurement by **using microplate reader**.
- **High correlation** with the result of Atomic Absorption Spectrometry (AAS).
- Sample pre-treatment is not required.
- Kit does not contain any hazardous components like cyanide or azide as preservative.
- 1 point calibration - do not need multiple point measurement to make standard curve ($r^2=0.9999$).
- The absorbance after coloring reaction is stable for at least 30 minutes.
- Lithium (Li^+) and Lithium Carbonate (Li_2CO_3) can be measured.
- You can get assay results just in **10 minutes** !
- All animal species samples can be measured.
- Patented technology : JAPAN : Patent. No.5100903, INTERNATIONAL : PCT/JP2012/61015



Assay Specification

- Samples : Serum, Plasma (Heparin Lithium contained samples cannot be used.)
- Assay range : 0.03 - 3.0 mM
- Measuring absorbance (Main / Correction) : 550 nm / 600 nm (600 nm - 610 nm)

Key points of this Assay



The absorbance after coloring reaction in proposed reagent is stable for at least 30 minutes.

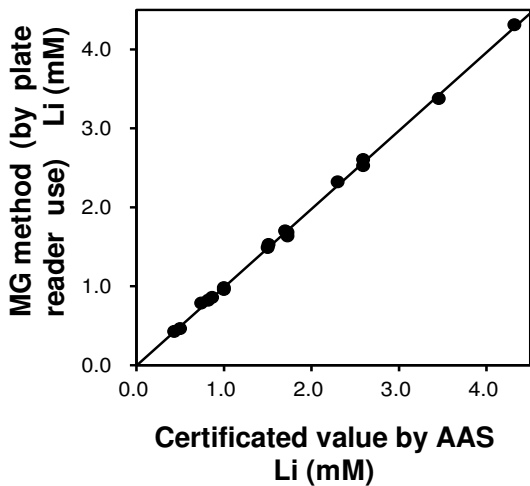
Wavelength: 546 nm (main), 600 nm (sub)

Sample : Li_2CO_3 added serum

Apparatus : CORONA SH-1200
(Micro-titer plate reader)

1-pointend method, at room temperature

N=3, S.D= less than 0.005 (Abs)



Good correlation between MG-method (Y) result and a obtainable method (X) result

Regression equation

$$Y = 0.992x - 0.005, r^2 = 0.9994$$

17 Control serum were used as specimen

【Roche】 Cfas II, Precinorm U, Precipath U,

【Sero】 Pathonorm H, Autonorm, Seronorm,

Seronorm Human,

Seronorm Pharmaca L1

Seronorm Pharmaca L2

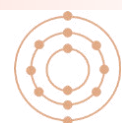
【Other】 Lithium added serum

(Serum base: Pathonorm L)

AAS : Atomic Absorption Spectrometry



Product Information



[Manufacturer : AKJ]

Product Name	Size	Catalog #	Storage
Li Lithium Assay kit LS - Polyfluoroporphyrin Method	100 tests	LI01ME	4°C

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ZF-X10T-03 (2014.10)

Oxidative Stress Assay Kit (TAC / Thiol)

For more information : http://www.funakoshi.co.jp/exports_contents/80379 (TAC), 80388 (Thiol)

Total Antioxidant Capacity Assay Kit

Reactive Oxygen Species (ROS) can cause damage to biomolecules such as DNA, proteins or lipids, and thought to be related to the development for cancer, Parkinson's disease, Alzheimer's disease, atherosclerosis, etc.

To protect from such damages, organisms produce and ingest various antioxidants that interact with and neutralize ROS.

Measurement of Total Antioxidant Capacity (TAC) is used as an integrated index rather than the simple sum of measurable antioxidants.

Metallogenics' TAC assay kit is compatible with wide range of samples.

Features

- Rapid assay (Just 10 minutes)
- Simple Protocol
- No hazardous components such as cyanide or azide as preservative.
- Compatible with multi-species
- Samples : Serum, Plasma, Urine, Foods such as wine, beverage and tea
- 2 points calibration

Principle

Antioxidants in the samples reduce Cu^{2+} to Cu^+ . Cu^+ is chelated by bathocuproine and yields an orange colored complex.

TAC can be determined by measuring absorbance of the generated complex at wavelength 490 nm (430 nm to 510 nm).

Data

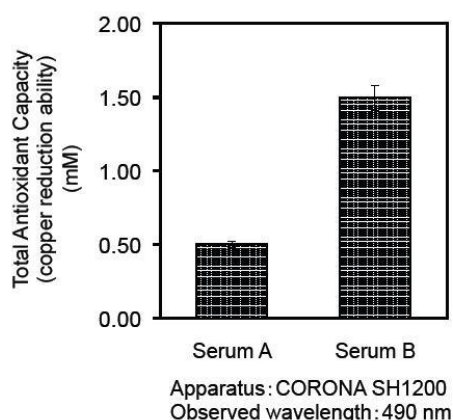


Figure : Measurement of TAC in bovine serum

(Note)

- Remove insoluble substances from serum and plasma samples by filtration or centrifugation.
- Hemolyzed sample cannot be used.
- Do not use EDTA as anticoagulant.
- Use same type of blood collection tubes when TACs in plasma samples are compared. (Some anticoagulant has reduction ability.)
- Fresh samples must be assayed. If you want to store the samples, keep them in -20°C and do not repeat freeze / thawing cycle that damage the samples.

Product Information

[Manufacturer : AKJ]

Product Name	Size	Code	Storage
Total Antioxidant Capacity Assay Kit (200 tests)	1 kit	AC01DE	4°C

Thiol Detection Assay Kit

In living organisms, thiol groups exist as free cysteine, glutathione, cysteine residues in proteins (such as albumin). Thiol groups attached to a carbon atom have a strong reducing ability, then plays an important role for the antioxidant capacity to scavenge ROS. According to a report, patients of various diseases exhibit low level of serum protein thiols. Furthermore, the oxidations of protein thiol groups contribute to the tertiary or quaternary structure of a protein.

Metallogenics' thiol detection assay kit (Ellman's method) measures total thiol groups in the sample. Ellman's reagent (DTNB: 5,5'-Dithiobis(2-nitrobenzoic acid)) reacts with thiol groups in the sample, and yields TNB2- (5-Mercapto-2-nitrobenzoic acid), showing yellow color. The intensity of the color is proportional to the thiol groups in the sample. Thiol concentration can be calculated by measuring absorbance of TNB2- at wavelength 412 nm (380 nm to 440 nm).

Features

- Rapid assay (Just 10 minutes)
- Simple Protocol
- No hazardous components such as cyanide or azide as preservative.
- Compatible with multi-species
- Samples : Serum, Plasma
- 2 points calibration

Data

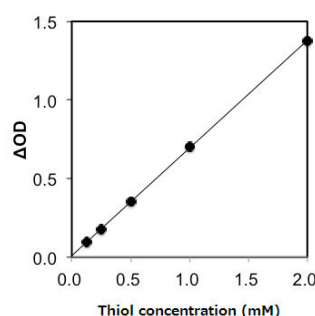


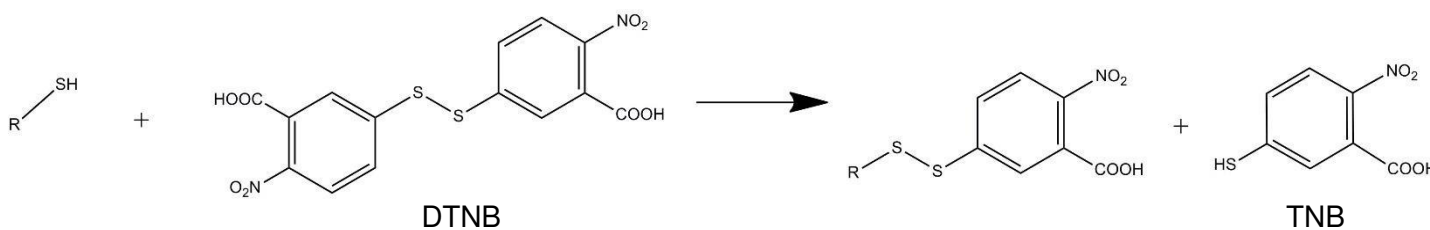
Figure : Standard curve

Principle

Ellman's method

Ellman's reagent (DTNB:5,5'-Dithiobis (2-nitrobenzoic acid)) reacts with thiol group and produces 5-Mercapto-2-nitrobenzoic acid (TNB).

Measure absorbance at $\lambda_{max}=412$ nm and quantify thiol concentration.



Product Information

[Manufacturer : AKJ]

Product Name	Size	Code	Storage
Thiol Detection Assay Kit (100 tests)	1 kit	TH01DE	4°C

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BioVerde

DMSO/Serum/Protein/Xeno-free StemCell Keep

For more information : http://www.funakoshi.co.jp/exports_contents/46011

DMSO-free

Serum-free

Protein-free

Xeno-free

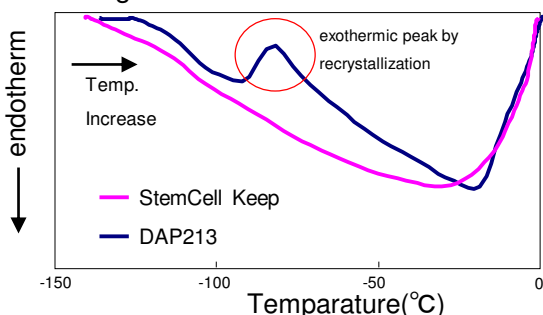
StemCell Keep is optimized cryopreservation media for primates ES/iPS cells by keeping high vitrification ability with low cyto-toxicity.

MEMO

Existent problem of preservation media contains DMSO and What is vitrification?

Very old method of cryopreservation was "Slow Programmed Freezing" using by 10% DMSO contained solution. However, big ice crystal is formed by this method and this crystal causes very poor cell viability (0.1-1%) for primate ES and iPS cells. Improved method is "vitrification" using by DAP213 (DMSO 2M, Acetamide 1M, Propylene glycol 3M).

Vitrification is the new method for preventing ice crystal formation by rapid freezing (<15 sec) in LN2. However, this method requires rapid thawing to prevent recrystallization and experienced operators. It is known that DMSO has influence on OCT-4 expression and differentiation, Acetamide is identified as carcinogens. Moreover, DAP213 is high osmolarity solution – it means this solution has high toxicity. Therefore, development of low Toxicity, easy to use vitrification solution had been expected. **StemCell Keep** is the solution that overcomes these disadvantages of DAP213!



Solution frozen by Liquid Nitrogen is heated by 50°C/min.

StemCell Keep does not have recrystallization during temperature rising.



Features

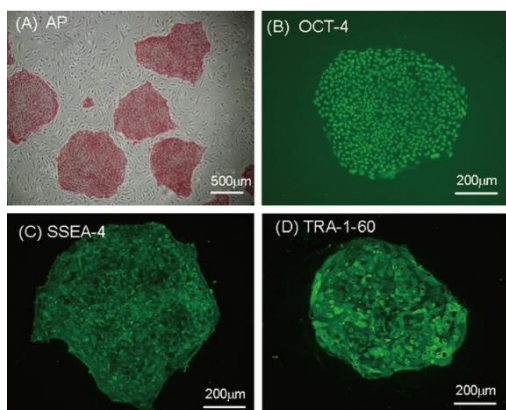
- Animal-derived Protein-free and DMSO-free formulation. No risk of differentiation effect by DMSO.
- Maintain ES and iPS cells with colony formation by vitrification.
- Maintain stem cell pluripotency after thawing.
- High cyto-protection effect and vitrification technology by new original cryoprotectant material is allowed to cryopreserve ES and iPS cell colonies.
- Sufficient for 100 vials.
- Free of bacteria, fungi and mycoplasma contamination.
- Long shelf life. The product is stable for 2 years at 4°C after the date of manufacture.

Trial Sample available!

Small size trial sample (5 mL) is available. Please contact your local distributors.

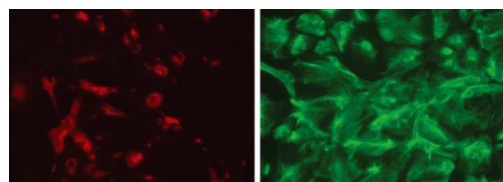
Experimental results and protocol are on next page.

Experimental Results



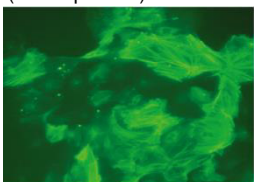
Differentiation of human iPS cells after cryopreservation with StemCell Keep.

Cells are positive for all of undifferentiation markers, Alkaline phosphatase (A), OCT-4 (B), SSEA-4 (C) and TRA-1-60 (D), and are maintained in the undifferentiated state.



Endodermal Cells
(α -Fetoprotein)

Ectodermal Cells (Nestin)



Mesodermal Cells (α -SMA)

Pluriipotency of human iPS cells after cryopreservation with StemCell Keep.

After preparation of embryoid bodies from human iPS cells, the cells are kept for up to 1 week in normal culture plate. Each of differentiation marker is detected.

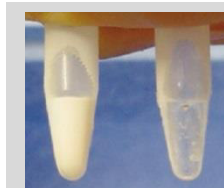
Protocol Outline

The preliminary experiment is necessary before the actual experiment is carried out.

Cryopreserving

Handling procedure video is available from :
http://www.funakoshi.co.jp/exports_contents/46011

1. Place a dewar flask filled with liquid nitrogen in the clean bench.
2. Detach ES / iPS cell colonies with 0.25% trypsin / 1 mg/ml collagenase IV / in PBS.
- ✗ One 60 mm dish of nearly confluent cells can be split into 1 - 5 vials.
3. Precipitate these cells by centrifugation and remove the supernatant . If several vials are needed for cryopreservation, they are stored in ice.
4. Suspend the cells with 200 μ l of StemCell Keep by pipetting. Close a lid of the cryopreservation vial, and transfer it to the dewar flask within 1 minute.
- ✗ If the vitrification is successful, the solution remains transparent.



Right: Vitrification is successful.
(Successful Example)
 Left: Recrystallization happened
(Failed Example)

5. Transfer the vial to the liquid nitrogen tank or -130°C deep freezer.

Thawing

1. Prepare each centrifugation vials each containing 9 ml of cell culture medium warmed at 37°C in water bath.
- ✗ Thaw one tube at a time. Leave others frozen.
2. Put one cryopreservation vial containing cells into a dewar flask filled with liquid nitrogen, and place it on clean bench.
3. Add 1 ml of warmed cell culture medium into the cryopreserved vial immediately, and then mix gently by pipetting.
4. Transfer the entire volume of diluted cells into the centrifugation vial, and centrifuge to wash cells.
5. After transfer the cells to the feeder plate, continue the further culture procedures according to standard protocols.

Product Information

[Manufacturer : BVD]

Product Name	Size	Catalog #	Storage
StemCell Keep	20 mL	VPL-A1	4°C

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ZF-Y02T-01 (2015.01)

2015

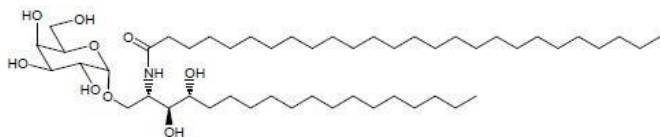
KRN7000

α – Galactosyl Ceramide

For more information :http://www.funakoshi.co.jp/exports_contents/46127

α -Galactosylceramide(α -Gal-Cer; KRN7000) was developed by Kyowa Hakko Kirin Company, as a biological response modifier. The α -Gal-Cer was isolated from an extract of the marine sponge, *Agelas mauritanus*, as an active substance which has galactose combined with ceramide in an α -configuration. α -Gal-Cer (KRN7000), a chemically synthesized α -Galactosylceramide, is a specific ligand for human and mouse natural killer T (NKT) cells. α -Gal-Cer exhibits potent antitumor activity in various kinds of *in vitro* murine experimental models including subcutaneously implanted model and metastatic models in the liver and lung. In the liver metastatic models, treatment with α -Gal-Cer suppressed the growth of tumors and prolonged the survival term of tumor-bearing mice. α -Gal-Cer has been reported to show various immunological influence in infectious disease, autoimmune disease, and graft versus host disease in mice.

Structure



For the research of...

- Anti-tumor response
- Anti-autoimmune response
- Anti-inflammation

through NKT – Dendritic Cells immune system

Citation

Chang CH *et al.*, PLoS One. 2015 Mar 25;10(3):e0121320.
Ando *et al.*, Oncol Rep. 2015 Feb;33(2):826-32.
Hasegawa *et al.*, Anticancer Res. 2014 Jul;34(7):3411-7.
Nakamura *et al.*, J Control Release. 2013 Oct 28;171(2):216-24
JJ O'Konek *et al.*, Clin Cancer Res. 2013 Aug 15;19(16):4404-11
Thomsen MK *et al.*, J Clin Invest. 2013 Dec;123(12):5258-68
Bjordahl RL *et al.*, PLoS One. 2012;7(8):e42635.

... and more!

Our KRN7000 is used on Proimmune's CD1d tetramers!



Note: Alpha-GalCer (KRN7000) is for research use only, not for use in human, therapeutic or diagnostic applications without the expressed written authorization of Kyowa Hakko Kirin Company.

Kyowa Hakko Kirin Company has issued a worldwide license for KRN7000 research to Funakoshi Company.

Product Information

[Manufacture : KRN]

Product Name	Size	Catalog #	Storage
α -Galactosylceramide < α -Gal-Cer>, <KRN7000>	1 mg	KRN7000	-20°C

2015

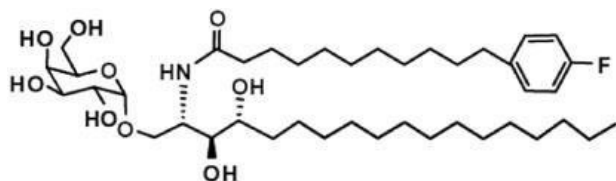
7DW8-5

 For more information : http://www.funakoshi.co.jp/exports_contents/46127

There are a number of glycolipids synthesized, some of which are analogs of α -galactosyl ceramide (α -Gal-Cer), a marine sponge lipid that is the most extensively studied CD1d ligand to date. These compounds were tested for their ability to stimulate human invariant natural killer T (iNKT) cell lines, secretion of key cytokines such as IFN- γ and IL-12, and activate autologous dendritic cells, as well as binding to CD1d and the invariant T-cell receptor. A lead compound 7DW8-5, emerged from these studies and this glycolipid exhibits a stronger adjuvant effect than α -Gal-Cer in various HIV vaccine platforms in mice. 7DW8-5 also provides a protective adjuvant effect with a candidate malaria vaccine when tested in mice infected with malaria parasites. While the majority of the studies performed focus on the potential of the glycolipids as a vaccine adjuvant, it is foreseeable that the compounds could also be used as a potential immunotherapeutic to treat cancer, infectious diseases, and autoimmune diseases.

※ This product is licensed by The Rockefeller University.

Structure



For the research of...

- Anti-tumor response
- Anti-autoimmune response
- Anti-inflammation

through NKT – Dendritic Cells immune system

Citation

- Li X, Fujio M, Imamura M, Wu D, Vasan S, Wong C-H, Ho DD, Tsuji M. 2010. Design of a novel CD1d-binding NKT cell ligand as a vaccine adjuvant. Proc. Natl. Acad. Sci. USA. 107: 13010-13015.
- Padte NN, Li X, Tsuji M, Vasan S. 2011. Clinical Development of a Novel CD1d-binding NKT Cell Ligand as a Vaccine Adjuvant. Clin. Immunol. 140: 142-151.

Product Information

[Manufacture : FRK]

Product Name	Size	Catalog #	Storage
7DW8-5	1 mg	7DW8-5	-20°C

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