



NutriScience Innovations, LLC

2450 Reservoir Avenue, Trumbull, CT 06611, U.S.A.

Tel: (203) 372-8877 | Fax: (203) 372-9977

e: Sales@NutriScienceUSA.com | w: NutriScienceUSA.com

BGF-IMMUNE® 1,3-BETA-GLUCAN 85%: COMPARISON OF BETA-GLUCANS

v.2015

Confidential, internal data.

These statements have not been evaluated by the Food and Drug Administration (FDA).

The product(s) on this site are not intended to diagnose, treat, cure, or prevent any disease.

BGF-Immune® is a registered trademark of NutriScience Innovations, LLC.

All material is property of NutriScience Innovations, LLC and shall not be duplicated, reproduced or used for any purpose without prior express written consent from NutriScience Innovations, LLC.

Materials and Methods

- Materials

- Raw 264.7 cell line, LPS, Endotoxin kit, MTS, PMS, NO kit, cDNA synthesis kit, PCR kit, Beta-Glucans

- Incubation time

- 24h, 37°C, 5% CO₂ incubation

Sample	Description
BGH	BGF-Immune® Beta-Glucan (1,3 linkage from fermentation)
YGW	Other branched chain Beta-Glucan (Yeast derived)

* "LPS" means Lipopolysaccharide.

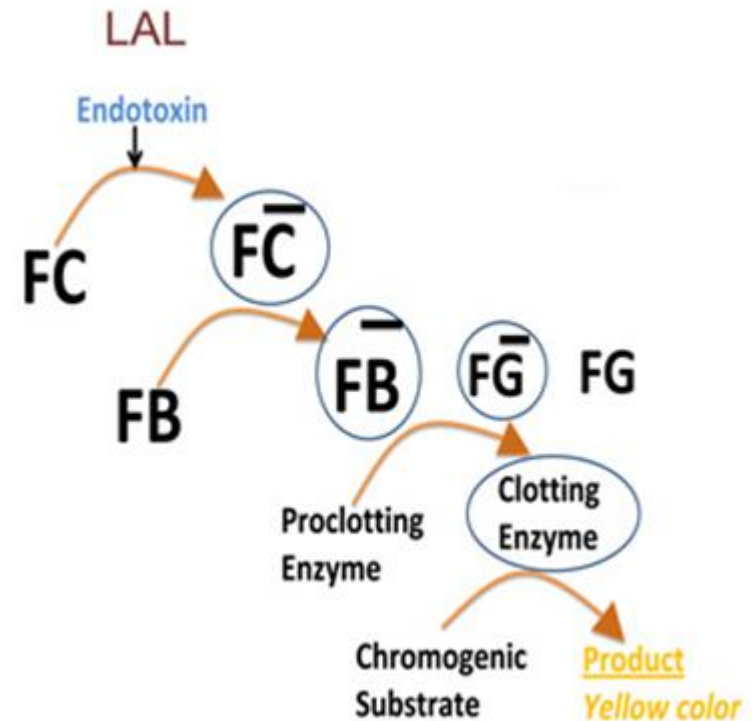
** "Cont." means control; tested without Beta-Glucan

- Concentration

- Prepare samples at 1% in DMSO sol'n
- Beta-Glucan conc. (µg/ml) : 10, 30
- LPS conc. : 200ng/ml

Endotoxin assay method

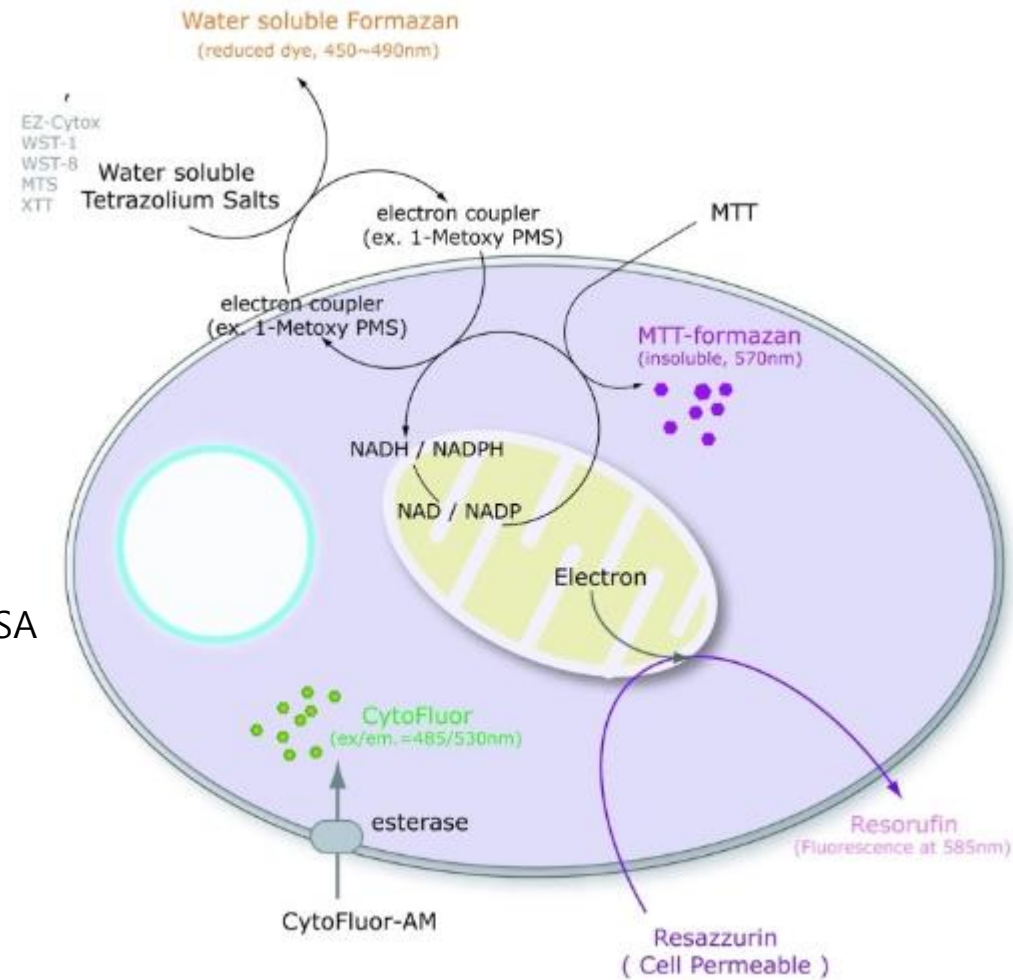
- ① Treatment of betaglucan to the cells
- ② Harvest the media in 24 hrs
- ③ Distribute samples on 96 wells
- ④ Distribute LAL lysate to the well
- ⑤ React at 37°C for 6min
- ⑥ Distribute a substrate
- ⑦ React at 37°C for 10min
- ⑧ Determine absorbance at 410nm with ELISA reader



Cell viability test

- Cell viability: MTS assay

- ① Raw264.7 cell is treated with betaglucan
- ② Removal of media in 24 hrs
- ③ Add MTS + PMS mixture
- ④ Incubate 1~4 hrs in a CO₂ incubator
- ⑤ Determine absorbance at 490nm with ELISA reader

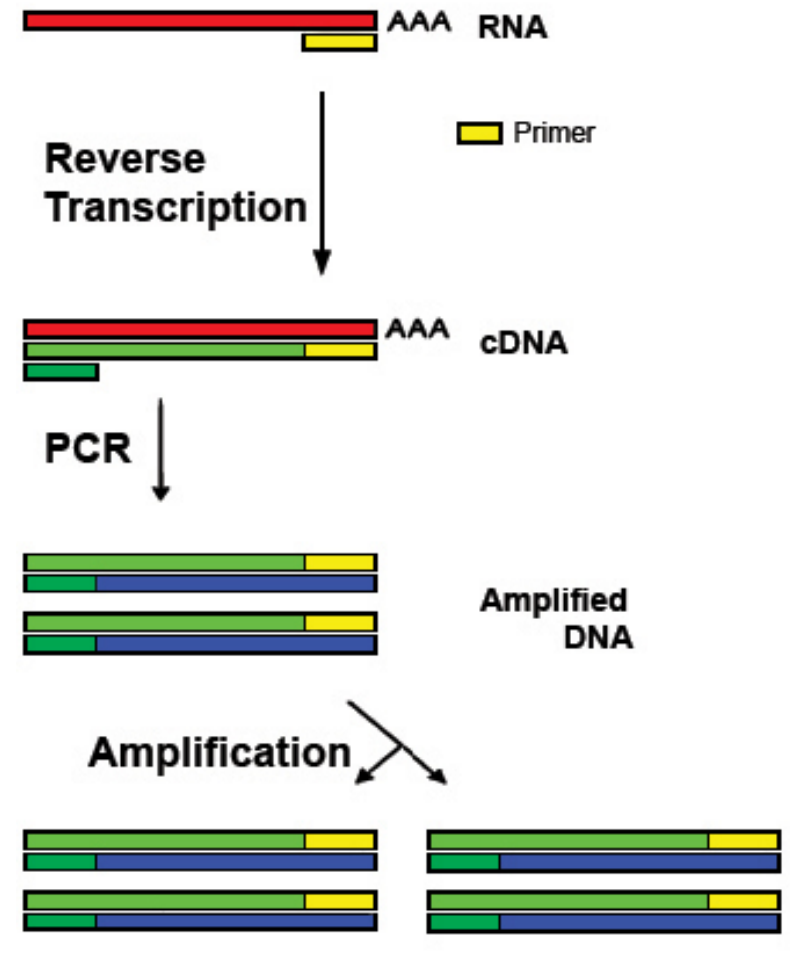


Live cell Assays

mRNA expression level assay for cytokines

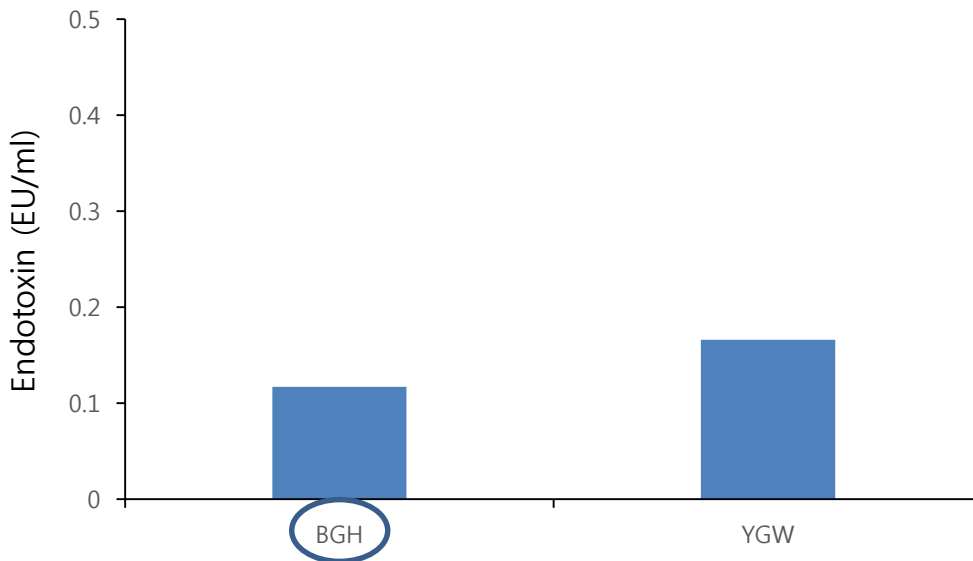
- Cytokine analysis by using RT-PCR

- ① Treat cells with betaglucan
- ② Remove the media in 24hrs
- ③ Extract RNA by the cell lysis
- ④ cDNA synthesis with the RNA
- ⑤ PCR with PCR kit
- ⑥ DNA determination on agarose gel



Toxicity test

<Endotoxin>

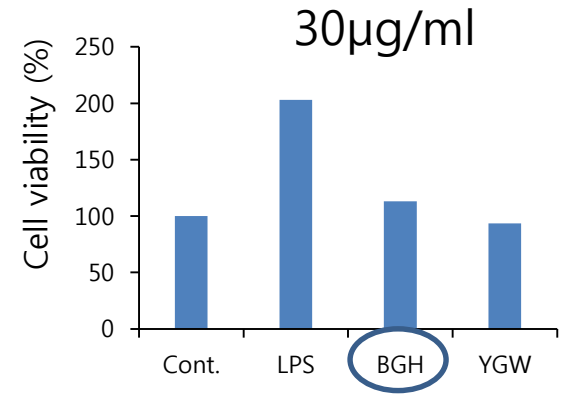
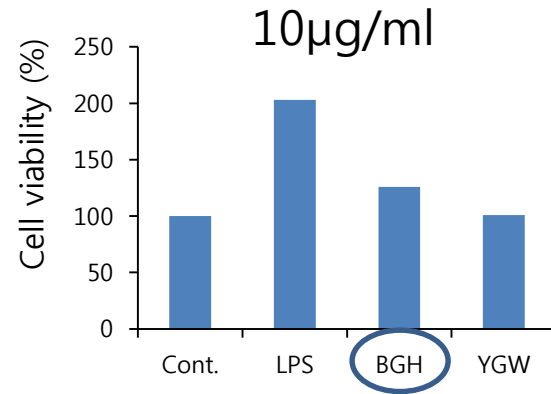
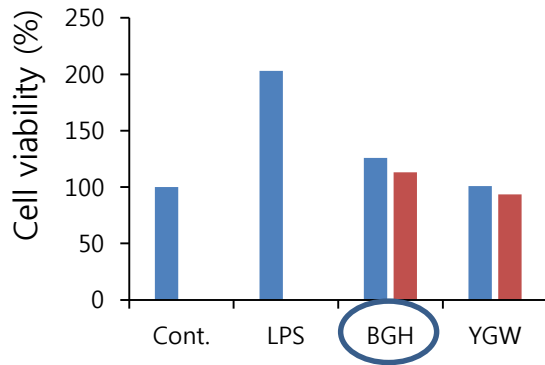


Sample	Endotoxin (EU/ml)
BGH	0.117
YGW	0.166

- Under USA FDA guideline, the endotoxin level of below 0.5 EU/ml is regarded as non-toxic.
Reference : FDA LAL Test Guideline (2009) Bacterial endotoxin test. USP 32 (85)

Cell Viability Test

<Cell viability>

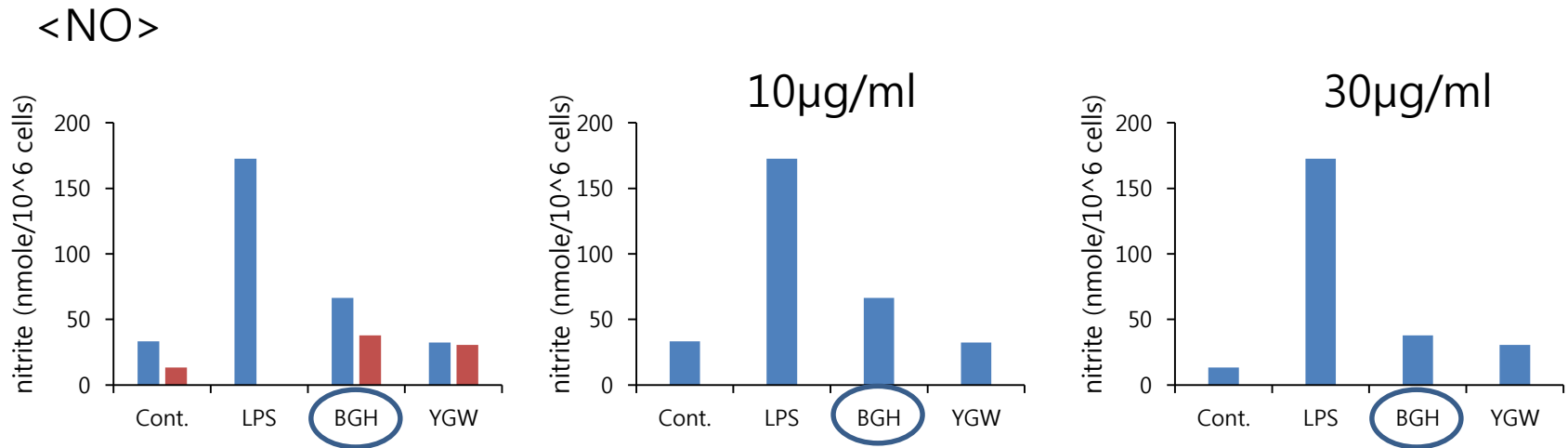


* "LPS" means Lipopolysaccharide.

** "Cont." means control; tested without Beta-Glucan

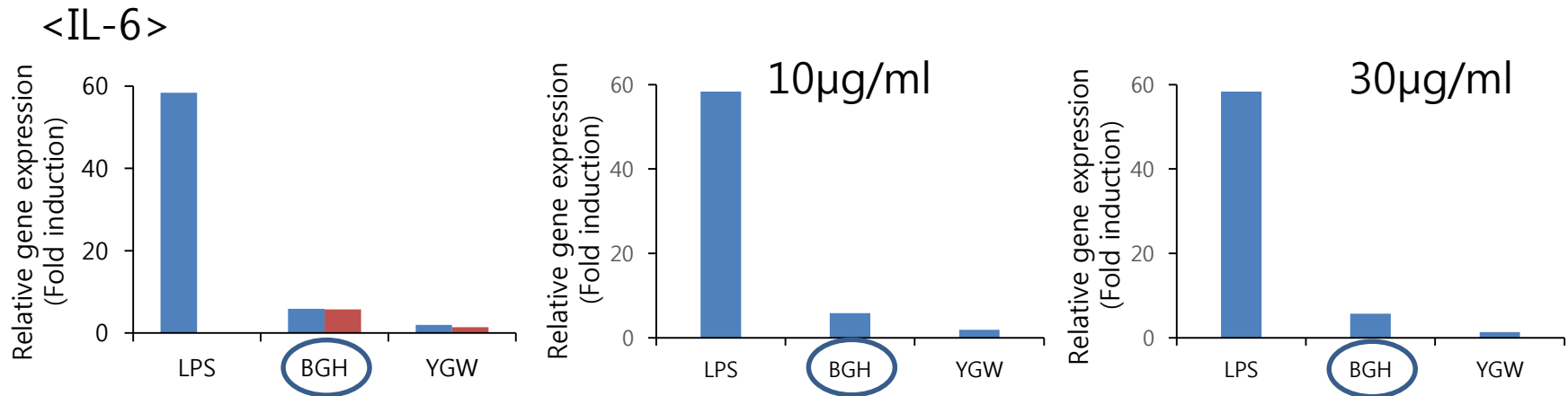
➤ BGH showed a little higher viability than YGW.

Nitric Oxide Formation



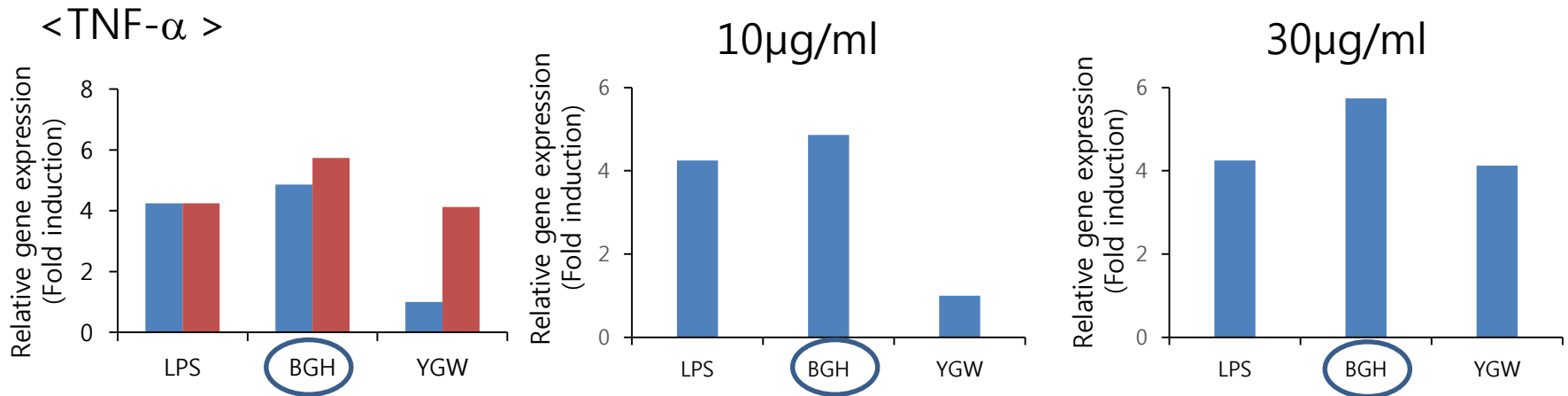
➤BGH showed a higher NO formation than YGW

IL-6 Gene Expression



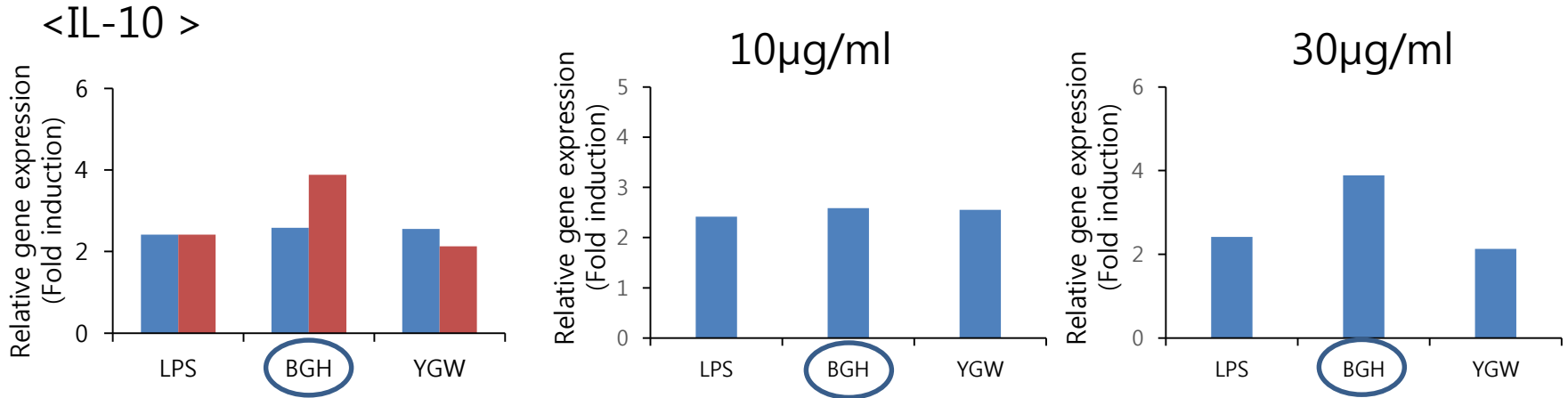
- IL-6 gene expression level with BGH was higher than that with YGW

TNF- α gene expression



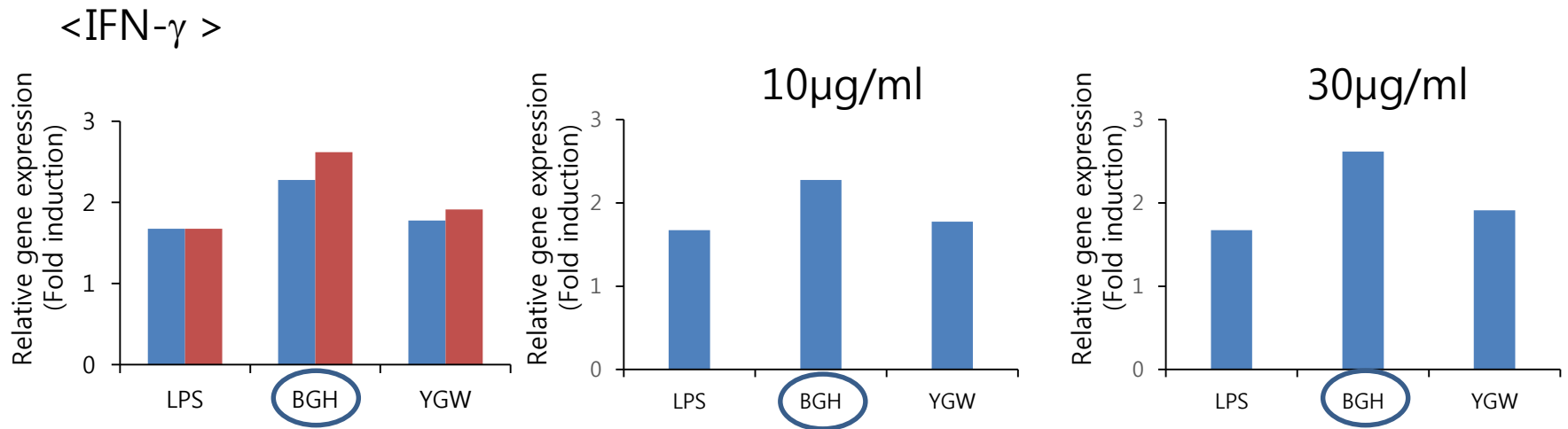
➤ TNF-alpha gene expression level with BGH was higher than that with YGW

IL-10 gene expression



➤ IL-10 gene expression level with BGH was higher than that with YGW

IFN- γ gene expression



➤ IFN- γ gene expression level with BGH was higher than that with YGW

Conclusions

- BGF-Immune® Beta-Glucan is not toxic to the normal cells
- As seen from data collected and evaluated, BGF-Immune® linear chain Beta-Glucan (1,3 linkage) shows a little higher gene expression level with immune related cytokines such as interferon-gamma, IL-6, IL-10 and TFF-alpha than the comparisons with branched Beta-Glucan from yeast.