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

v.2015
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% CO\textsubscript{2} incubation

<table>
<thead>
<tr>
<th>Sample</th>
<th>Description</th>
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<tbody>
<tr>
<td>BGH</td>
<td>BGF-Immune® Beta-Glucan (1,3 linkage from fermentation)</td>
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<tr>
<td>YGW</td>
<td>Other branched chain Beta-Glucan (Yeast derived)</td>
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• **Concentration**
  - Prepare samples at 1% in DMSO sol’n
  - Beta-Glucan conc. (μg/ml) : 10, 30
  - LPS conc. : 200ng/ml

*“LPS” means Lipopolysaccharide.*
**“Cont.” means control; tested without Beta-Glucan**
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
Nitric Oxide assay

- **Nitric Oxide**
  1. Treatment cells with betaglucan
  2. Harvest the media in 24hrs
  3. Distribute samples on 96wells
  4. Add sulfanilamide, then incubate samples for 10~15 min
  5. Add NED, then incubate it in a dark room for 10~15min
  6. Determine absorbance at 520~550nm with ELISA reader
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
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)
BGH showed a little higher viability than YGW.

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Nitric Oxide Formation

BGH showed a higher NO formation than YGW
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 level with BGH was higher than that with YGW
IFN-\(\gamma\) 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.