Unique cell-free protein synthesis system, PUREfrx
- Useful platform for protein expression in the development of biologics -

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Abstract

PUREfrx® is a cell-free protein synthesis system based on the PURE (Protein synthesis Using Recombinant Elements) system. PURE system is a reconstituted cell-free protein synthesis system, which consists of only purified factors necessary for transcription, translation and energy regeneration. The PURE system has the unique features: It contains less contaminant such as nucleases and proteases, and the composition of the reagents can be easily adjusted in accordance with the purpose. We refined the preparation methods of all components that were purified from E. coli and developed the new PURE system as “PUREfrx®”. The latest version of PUREfrx® is PUREfrx® 2.0, which has the productivity of CPP and E. coli dihydrofolate reductase reaching to approximately 1 mg/mL in simple batch mode. The product is easily purified by simple method, and also it is directly applicable to cell-based assay even without purification because of very few endotoxin level. Here we report three topics about PUREfrx® 2.0 and PUREfrx® 2.2.

1. AT-rich codon at the N-terminal region of ORF facilitates the productivity in various proteins including proteins containing disulfide bonds and membrane proteins.
2. Reducing reagents in the reaction mixture have influence on the formation of disulfide bonds in the synthesized protein.
3. Functional glycosylated IgGs such as Trastuzumab and Nivolumab could be synthesized with a productivity of 30-120 μg/mL under the optimized condition.

**PUREfrx®; based on the PURE system technology**

The PURE system is a reconstituted cell-free protein synthesis system, which consists of only purified factors necessary for transcription, translation and energy regeneration.

**PUREfrx® 2.0**

- Regular kit for the protein synthesis containing DTT as a reducing reagent
- Regular kit for the protein synthesis capable of selecting a reducing reagent
- DS supplement
- Supplement for the synthesis of proteins containing disulfide bonds
- DNA Mix / GroMix

**PUREfrx® 2.2**

- Regular kit for the protein synthesis containing DTT as a reducing reagent
- Regular kit for the protein synthesis capable of selecting a reducing reagent
- DS supplement
- Supplement for the synthesis of proteins containing disulfide bonds
- DNA Mix / GroMix

**E. coli-based cell-free protein synthesis system**

<table>
<thead>
<tr>
<th>Extract system</th>
<th>Recombinated system</th>
</tr>
</thead>
<tbody>
<tr>
<td>S30 system</td>
<td>PURE system (original)</td>
</tr>
<tr>
<td>Typical Yield (μg/mL)</td>
<td>1-4,000</td>
</tr>
<tr>
<td>Contamination</td>
<td>RNAase</td>
</tr>
<tr>
<td></td>
<td>DNAase</td>
</tr>
<tr>
<td>Template DNA</td>
<td>Plasmid DNA</td>
</tr>
<tr>
<td></td>
<td>PCR product (from 3’-UTR)</td>
</tr>
<tr>
<td>Customization of composition</td>
<td>Difficult</td>
</tr>
<tr>
<td>Purification of His-tagged product</td>
<td>OK</td>
</tr>
</tbody>
</table>

**1. N-terminal codon and protein synthesis**

Trastuzumab Fab HC

<table>
<thead>
<tr>
<th>Frequency (%)</th>
<th>57</th>
<th>25</th>
<th>18</th>
<th>2</th>
<th>1</th>
</tr>
</thead>
</table>

- AT-rich codon > Major codon @ 5’-terminus of ORF

**2. Synthesis of proteins containing disulfide bonds**

2-1. Alkaline phosphatase (2 disulfide bonds)

**PUREfrx® 2.1**

- Containing DTT or CDB
- Sodium phosphate (50mM) and indicated concentrations
- Alkaline phosphatase (1U/mL)
- Incubation at 37°C for 1 hour
  
a) SDS-PAGE under reducing and non-reducing condition
  b) Activity assay

Reducing reagents influence on the formation of correct disulfide bonds within the synthesized polypeptide.

2-2. Acid phosphatase (5 disulfide bonds)

**PUREfrx® 2.2**

- Containing DTT or CDB
- 1U alkaline phosphatase (1U/mL)
  
a) SDS-PAGE under reducing and non-reducing condition
  b) Activity assay

2-3. IgG (Trastuzumab) (14 disulfide bonds)

**PUREfrx® 2.3**

- Containing indicated reducing reagent
- 1U alkaline phosphatase (1U/mL)
- Sodium phosphate (100mM) and indicated concentrations
  
a) SDS-PAGE under reducing and non-reducing condition
  b) Activity assay

**3. Synthesis of functional glycosylated IgG**

**PUREfrx® 2.4**

- Containing CDB or CDB
- 1U alkaline phosphatase (1U/mL)
- Sodium phosphate (50mM) and indicated concentrations
  
a) SDS-PAGE under reducing and non-reducing condition
  b) ELISA

- GC content (%) | 384 | Tested clones | 50 |

<table>
<thead>
<tr>
<th>Key Factors for IgG synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>- H-LUC DNA ratio (1:1.2)</td>
</tr>
<tr>
<td>- Redox state (COS/COSC)</td>
</tr>
<tr>
<td>- Molecular shape (DNA, -Dox)</td>
</tr>
<tr>
<td>- Incubation temperature (37°C)</td>
</tr>
<tr>
<td>- Incubation Time (0-24 hours)</td>
</tr>
</tbody>
</table>

**Summary of synthesis of various IgGs using PUREfrx®**

<table>
<thead>
<tr>
<th>Name</th>
<th>Fab</th>
<th>Human DTV</th>
<th>Human DNA</th>
<th>Specific activity (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-HER2 IgG Fab</td>
<td>37</td>
<td>4.2×10⁴</td>
<td>2.4×10⁴</td>
<td>100</td>
</tr>
<tr>
<td>Anti-HER2 IgG</td>
<td>37</td>
<td>4.2×10⁴</td>
<td>2.4×10⁴</td>
<td>100</td>
</tr>
<tr>
<td>Anti-HER2 IgG 1C3</td>
<td>37</td>
<td>4.2×10⁴</td>
<td>2.4×10⁴</td>
<td>100</td>
</tr>
<tr>
<td>Anti-HER2 IgG 2A10</td>
<td>37</td>
<td>4.2×10⁴</td>
<td>2.4×10⁴</td>
<td>100</td>
</tr>
</tbody>
</table>

**Functional glycosylated IgG can be synthesized using PUREfrx® under the optimized condition.**

For more information, please contact us.
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