作为开发新型实用性人绒毛膜促性腺激素（C-FG）疫苗的一种尝试，我们已构建若干组合靶抗原三个线性H1细胞表位和外源强71细胞表位的基因工程C-FG嵌合肽。为了检测用这些嵌合肽免疫的动物血清中是否能产生抗各表位的三种抗体，本研究选用能在大肠杆菌中高表达和与生物素亲和性强且特异（方便通过亲和层析纯化）的链霉亲和素为载体，分别构建了三种含1C-FG不同单一线性H1细胞表位（5，3和4）的融合蛋白。在链霉亲和素基因下游多克隆区30（I和4#E位点插入各表位编码基因片段（带7JJ终止密码子）的@7KJ1#4重组质粒，转
化HL!#（M90）#L/?K宿主菌后，它们在NO7G诱导下均能以较高水平表达各自目的融合蛋白，而且它们的表达产物在P8?A8.，Q'&A鉴定中都能被抗各表位特异的多抗或单抗或抗报告表位单抗识别。用改良的制备性OJG9方法可
以一步纯化电泳均一性高于35R的三个融合蛋白，它们的收得率相对#L培养物约为5 :S。作为化学合成表位肽的替代物，1C-FG三个单一H1细胞表位融合蛋白的可获得性将有助于所构建C-FG基因工程嵌合肽以及其他C-FG疫苗，也包括它的M)J疫苗的免疫原性分析。
Table 1 The sense strand sequences of β5 β9 and β8 epitopes of β-hCG

<table>
<thead>
<tr>
<th>Epitope</th>
<th>Amino acid sequence</th>
<th>Synthetic oligonucleotides</th>
</tr>
</thead>
<tbody>
<tr>
<td>β5</td>
<td>LQGVLPA</td>
<td>5'-TTGCCAAGGTGTGCTGCTTTG-3'</td>
</tr>
<tr>
<td>β9</td>
<td>PRFQ</td>
<td>5'-CTAGATTTCCAAA-3'</td>
</tr>
<tr>
<td>β8'</td>
<td>RLPSDTPLI</td>
<td>5'-AGATGCCAGGTCATCCGACATCCATGCTGCA-3'</td>
</tr>
</tbody>
</table>

Note: The β8' sequence includes a reporter sequence that can be recognized by mAb OT3A.

1.2

1.2.1 DNA 6 DNA

1.2.2 BL21/pTSA18/Stv-β5 BL21/pTSA18/Stv-β9 BL21/pTSA18/Stv-β8

1.2.3 β-hCG PAGE

1.2.4 β-hCG PAGE

2

2.1 β-hCG DNA

2.2 β-hCG PAGE

Sano
诱导下可高表达对生物素具有强且特异亲和性的链霉亲和素蛋白，也可与其他靶蛋白重组表达目的融合蛋白。在本研究中，用 IPTG 诱导后，插入片段引入终止密码子的终止密码子，融合基因都获得了特异性高表达，而且在凝胶上它们各自的表达产物所显示的分子量与各自约 3-4 倍的理论分子量一致（图 3）。但是，为比较两者表达水平而未引入终止密码子的另一组，各融合基因（多编码 66 个）在同样的诱导条件下，在凝胶上却未能辨识出它们特异蛋白的表达，用各特异多抗和单抗的免疫印迹检测证实了它们在大肠杆菌中确实未表达。

结果经制备性一步纯化可获取各约 25% 的融合蛋白。各纯化蛋白的分析见图 4。

### 2.3 Stv-β5* / Stv-β9* / Stv-β8* 融合蛋白的表达和纯化

![Western blot](https://example.com/western_blot)

**Fig. 2** Western blot of expressed Stv-β5* / Stv-β9* / Stv-β8* fusion proteins

M[ protein molecular weight marker[ ] 1] un-induced BL21/pTSA18-Stv-β5*[ ] 2 – 4 ■ induced BL21/pTSA18-Stv-β5*□ / pTSA18-Stv-β9+ and / pTSA18-Stv-β8*[ ] 5 ■ un-induced BL21/pTSA18-Stv-β5*□ / pTSA18-Stv-β9+ and / pTSA18-Stv-β8*[ ] 6 – 8 ■ induced BL21/pTSA18-Stv-β5*□ / pTSA18-Stv-β9+ and / pTSA18-Stv-β8*[ ]. Note■ + and – symbols indicate the presence or absence of TAA codon in the inserted target β epitope fragments.

![SDS-PAGE](https://example.com/sds_page)

**Fig. 1** SDS-PAGE analysis of six Stv-βE fusion proteins expressed in E. coli

M[ protein molecular weight marker[ ] 1] un-induced BL21/pTSA18-Stv-β5*[ ] 2 – 4 ■ induced BL21/pTSA18-Stv-β5*□ / pTSA18-Stv-β9+ and / pTSA18-Stv-β8*[ ] 5 ■ un-induced BL21/pTSA18-Stv-β5*□ / pTSA18-Stv-β9+ and / pTSA18-Stv-β8*[ ] 6 – 8 ■ induced BL21/pTSA18-Stv-β5*□ / pTSA18-Stv-β9+ and / pTSA18-Stv-β8*[ ]. Note■ + and – symbols indicate the presence or absence of TAA codon in the inserted target β epitope fragments.

### 2.4 Stv-β5* / Stv-β9* / Stv-β8* 融合蛋白的表达和纯化

![Western blot](https://example.com/western_blot)

**Fig. 2** Western blot of expressed Stv-β5* / Stv-β9* / Stv-β8* fusion proteins

M[ protein molecular weight marker[ ] 1] un-induced BL21/pTSA18-Stv-β5*[ ] 2 – 4 ■ induced BL21/pTSA18-Stv-β5*□ / pTSA18-Stv-β9+ and / pTSA18-Stv-β8*[ ] 5 ■ un-induced BL21/pTSA18-Stv-β5*□ / pTSA18-Stv-β9+ and / pTSA18-Stv-β8*[ ] 6 – 8 ■ induced BL21/pTSA18-Stv-β5*□ / pTSA18-Stv-β9+ and / pTSA18-Stv-β8*[ ]. Note■ + and – symbols indicate the presence or absence of TAA codon in the inserted target β epitope fragments.

- HSD-hCG
- β-hCG
- OT3A
- β-antibody
- β-antibody
- β-antibody
- β-antibody
- β-antibody
- β-antibody
- β-antibody
- β-antibody
- β-antibody
- β-antibody
- β-antibody
- β-antibody

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Fig. 3  SDS-PAGE analysis of purified Stv-βE fusion proteins M. Protein molecular weight markers 1, 4 and 7. Induced BL21/pTSA18-Stv-βE or Stv-βE 5 and 8. Each inclusion body proteins containing Stv-βE or Stv-βE fusion protein 3, 6 and 9. Purified Stv-βE or Stv-βE fusion protein.

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REFERENCES

Expression and Purification of Three Fusion Proteins Containing a Single B-cell Epitope β5β9 or β8β9 of Human Chorionic Gonadotropin β Subunit

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Abstract The possibility of using a subunit or fragment of human chorionic gonadotropin hCG as an immunogen for birth control has been actively explored for many years. This protein hormone is produced by the fertilized egg and is required for implantation of the blastocyst into the maternal uterus and the maintenance of pregnancy. In previous studies several bio-synthesized hCG chimeric peptides CFP that contain three linear B- cell epitopes β5β9 and β8β9 of β-hCG subunit together with various foreign ‘promiscuous’ T- cell epitopes were constructed and expressed as potential new hCG vaccine immunogens. In order to detect antibodies to each of the individual B-cell epitopes present in the animal antiserum raised against the hCG CFP we decided to construct three recombinant proteins each contains a single target B- cell epitope β5β9 or β8β9 of β-hCG. Two sets of DNA fragments were chemically synthesized encoding the β5β9 and β8β9 epitopes β12β12 45~52 113~116 or 133~144 of β-hCG subunit and were inserted into the downstream of streptavidin Stv gene in pTSA18 separately with or without an extra TAA codon at the 3’-terminals of the genes. SDS-PAGE analysis revealed that only Stv-β5β9 or Stv-β8β9 fusion genes set with the TAA codon can be expressed in E. coli B12 DE3 pLysS strain at high level after 1mM IPTG induction for 4 hours. Additionally these fusion proteins can all be recognized by specific polyclonal antiserum RS-4157 which was generated upon immunization with the loop peptide 38~57 of β-hCG monoclonal antibodies mAbL FB12 to β9 epitope and mAb OT3A that specially recognizes reporter sequence 133~139 of β8 epitope 137~144. Each of the proteins can be purified to 95% relative homogeneity using an improved method of preparative gel polyacrylamide gel electrophoresis. The yields were 5 mg per 1L culture. The three target Stv-βE fusion proteins will be useful in determining the immunogenicity of designed hCG GP and hCG vaccines including hCG DNA vaccines.

Key words β-hCG subunit B-cell epitopes streptavidin fusion expression purification

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