Producing Recombinant Adenovirus Encoding Green Fluorescent Protein Ad-GFP by Suspension Cultured HEK-293 N3S Cells

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Abstract Adenovirus vectors are one of the most promising gene transfer systems. They are of great value for gene therapy because these vectors achieve temporal high-level transgene expression and high gene transfer efficiency. To meet increasing needs of adenovirus vectors for gene therapy programs, parallel development of efficient, scalable, and reproducible production processes is required. Perfusion cultivation of 293 cells is one of the most commonly used methods to produce adenovirus vectors and it is suitable for industrialized production specially. Experimental studies had been carried out to produce recombinant adenovirus containing the green fluorescent protein gene Ad-GFP by perfusion cultivation of HEK-293 N3S cells in a 5L stirring bioreactor. Perfusion rate was 1 ~ 2 volume/day. To infect the 293 N3S cells with Ad-GFP at the density of 2 ~ 4×10⁶ cells/mL. The time of collecting cells was 48 hours post infection. After three rounds of freeze/thaw and centrifugation the crude viral lysates were stored at −80 °C until use. Then to get the Ad-GFP products by 2×CsCl-gradient purification. The purity of the...
products was determined by the $A_{260}/A_{280}$ ratio and a high performance liquid chromatography HPLC assay. The infective titer was determined by a TCID$_{50}$ assay. The culture term was 10 ~ 12 days. The infectious titer number of virus particle and the ratio of infectious titer to virus particle for the product were $1.0 \times 10^{11}$ IU/ml, $1.68 \times 10^{12}$ VP/mL and 6.0% IU/VP respectively. The $A_{260}/A_{280}$ ratio was 1.33 and the purity determined by HPLC was 99.2%. The cell specific productivity was around 1000IU/cell. By perfusion cultivation of 293 N3S cells in a 5L stirring bioreactors we established the production process for Ad-GFP which paves a way to produce other recombinant adenovirus for gene therapy.

**Key words** suspension culture recombinant adenovirus HEK-293 N3S cells bioreactor

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1.1

HEK-293 N3S

American Type Culture Collection ATCC 293 N3S

1.2

Baxter Healthcare

Corp. Gene Therapy Unit

1.3

DMEM/F12 GIBCO

1:1 pH 7.2 FBS

5%

1.4

MCS-1041 Techne B. Braun Biotech

5L 4L B. Braun Biotech

4L pH 7.2

250ml 500ml 1000ml

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pH 10.4

3mm 3.7mm

75um

Waters BECKMAN DU640

1.5

5L

37°C pH 7.0 3L/min

2 ~ 5 $\times 10^5$ /mL

1 $\times 10^6$ /mL

Vol/mL $	imes 10^6$ /mL

$\times 10^6$ /mL

Vol/mL

2 $\times 10^6$ /mL

20 MOI

multiplicity of infection

Ad-GFP

48h

$\times 10^6$

80°C

1.6

3

CsCl

CsCl

CsCl

1.7

1.7.1

3

1.7.2

$A_{260}/A_{280}$

HPLC

$A_{260}/A_{280}$

HPLC

SOURCE 15Q

4.6mm 100mm

Tris-HCl pH8.0 B 1 mol/L NaCl 20mmol/L Tris-

pH8.0 A $\rightarrow$ B 0$\rightarrow$ 40min

0.6mL/min 260mm

100mL
法计算纯度

电泳检测腺病毒产品蛋白图谱，上样考马斯亮蓝染色。

病毒感染滴度测定：采用组织培养半数感染剂量法（）对培养上清、细胞裂解液和纯化病毒进行感染滴度测定。

结果

磁力搅拌瓶内的细胞生长情况

细胞接入磁力搅拌瓶内进行流加培养，接种细胞密度为（），初始培养体积为，搅拌速度为。此后每计数细胞密度和细胞活力，绘制细胞生长曲线如图，细胞活力都在以上。当细胞密度达到（）时将细胞悬液接入生物反应器内。

图1 磁力搅拌瓶细胞生长曲线

图2 生物反应器细胞生长曲线

的纯化及纯度测定

经两步超速离心法纯化的病毒产品无色透明，无肉眼可见杂质。

比值，病毒颗粒数（根据计算公式：）结果见表。

表1 两步CsCl-gradient purification

<table>
<thead>
<tr>
<th></th>
<th>$A_{260}$</th>
<th>$A_{280}$</th>
<th>$A_{260}/A_{280}$</th>
<th>The number of virus particles VP/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A_{260}$</td>
<td>0.31 ± 0.04</td>
<td>0.23 ± 0.02</td>
<td>1.33 ± 0.03</td>
<td>1.68 ± 0.25 × 10^{12}</td>
</tr>
</tbody>
</table>

图3 HPLC chromatogram of recombinant adenovirus products

图4 SDS-PAGE analysis of recombinant adenovirus products

Ad-GFP

图5 SDS-PAGE分析重组腺病毒产品的电泳图谱

田博等：悬浮培养HEK-293 N3S 细胞生产重组腺病毒Ad-GFP
细胞密度效应。这种现象严重影响了腺病毒的产

度比较恒定，倍增时间在

希望提高产量。收集时间选择及优化是重要环节。

际,认为细胞密度在

差,此时感染病毒产率较低,所以流加培养难以满足

细胞,病毒尚处于复制阶段,没有达到最大复制量;

性,维持细胞最快生长速度又能降低成本,我们认为细

乳酸和氨)的浓度,使细胞维持在一个相对稳定的环

的灌流培养,将细胞密度提高到

换、金属螯合、凝胶过滤等,以替代传统的氯化铯纯

种情况: 1) 若收集过早,则细胞密度增加而病毒产量反而降低,即所谓的

乳酸和氨)的浓度,使细胞维持在一个相对稳定的环

的培养基方法可以部分减轻细胞密度效应的影响,我

差,此时感染病毒产率较低,所以流加培养难以满足

大规模培养的要求。连续灌流培养通过不断

提高了细胞密度,该技术自

后发现,细胞密度维持在

细胞培养实验后发现,细胞密度达到

倍,但当达到

由此,我们需要进一步探索不同灌流率对细胞密度和病毒产

态变差,死细胞聚集呈片状。虽然转瓶培养也能

面的消耗,此时收获细胞产量为

化方法

生物反应器内,优化了细胞培养环境,从而可达到较高的细胞

1 × 10^3 ~ 1 × 10^6 /mL。当细胞密度达到

表达产物,同时应用细胞截留装置使细胞保留于生物反应

度的灌流培养,将细胞密度提高到

（中国肿瘤生物治疗杂志）,进一步扩大生产规模奠定了基

细胞在接入反应器后的

乳酸和氨)的浓度,使细胞维持在一个相对稳定的环

表

<table>
<thead>
<tr>
<th>灌流速率</th>
<th>Maximum productivity</th>
<th>Parameters</th>
<th>Conditions</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>0.8</td>
<td>Cell Density</td>
<td>24h</td>
<td>3 × 10^6 /mL</td>
</tr>
</tbody>
</table>

**REFERENCES**