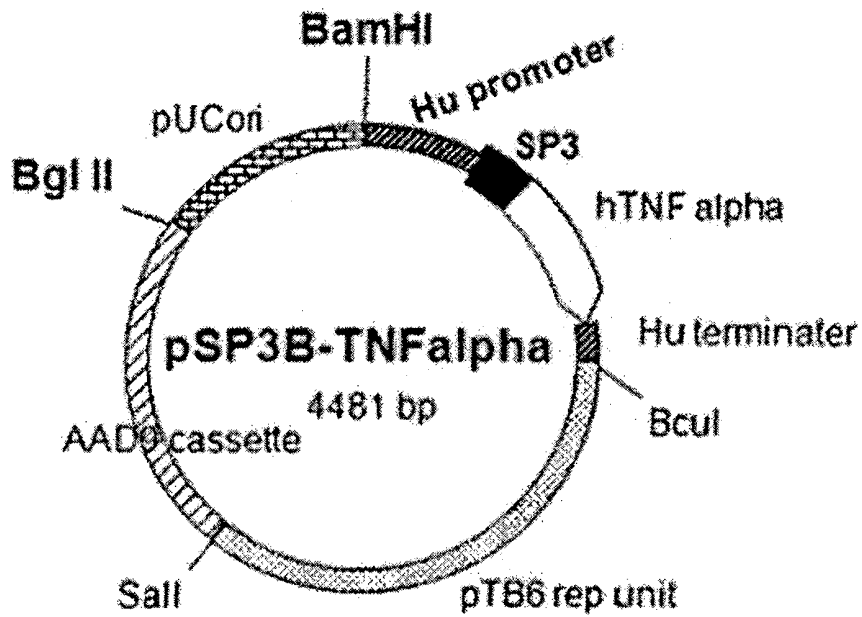
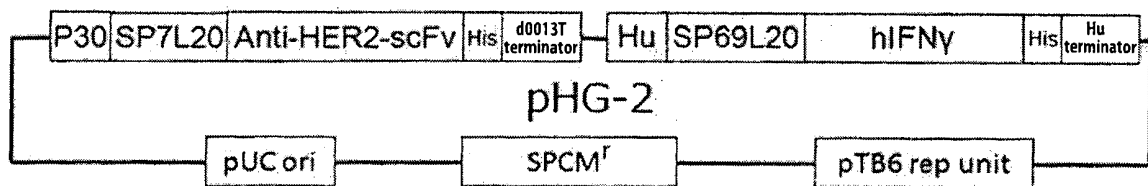


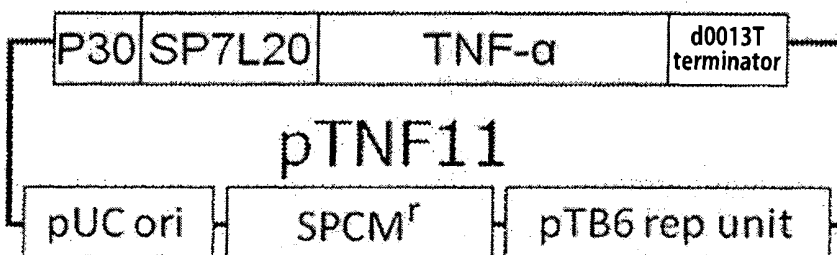
[図1]



[図2]

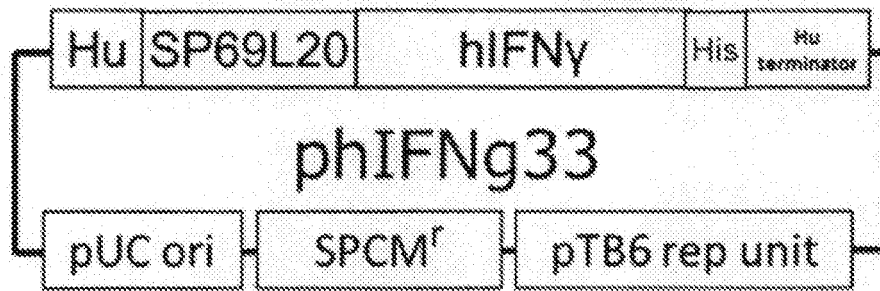


[図3]

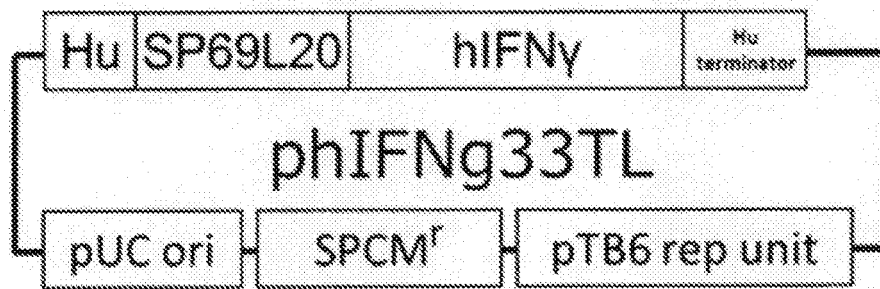


[図4]

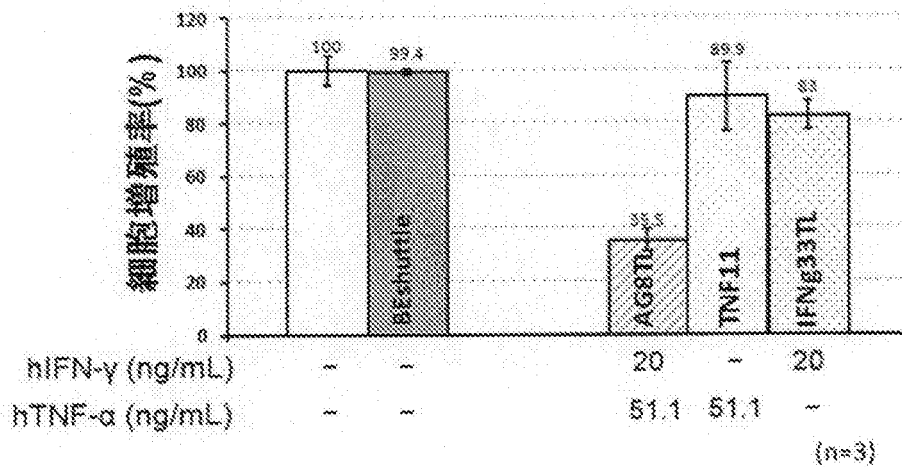
(a)



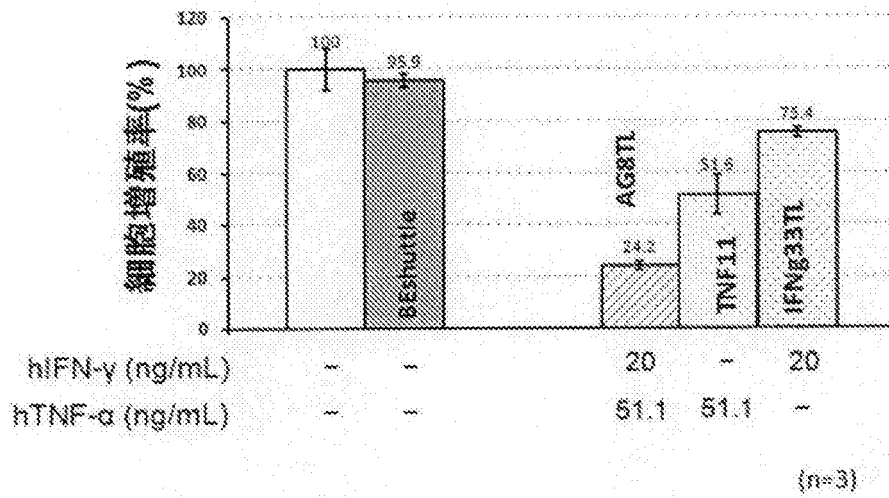
(b)



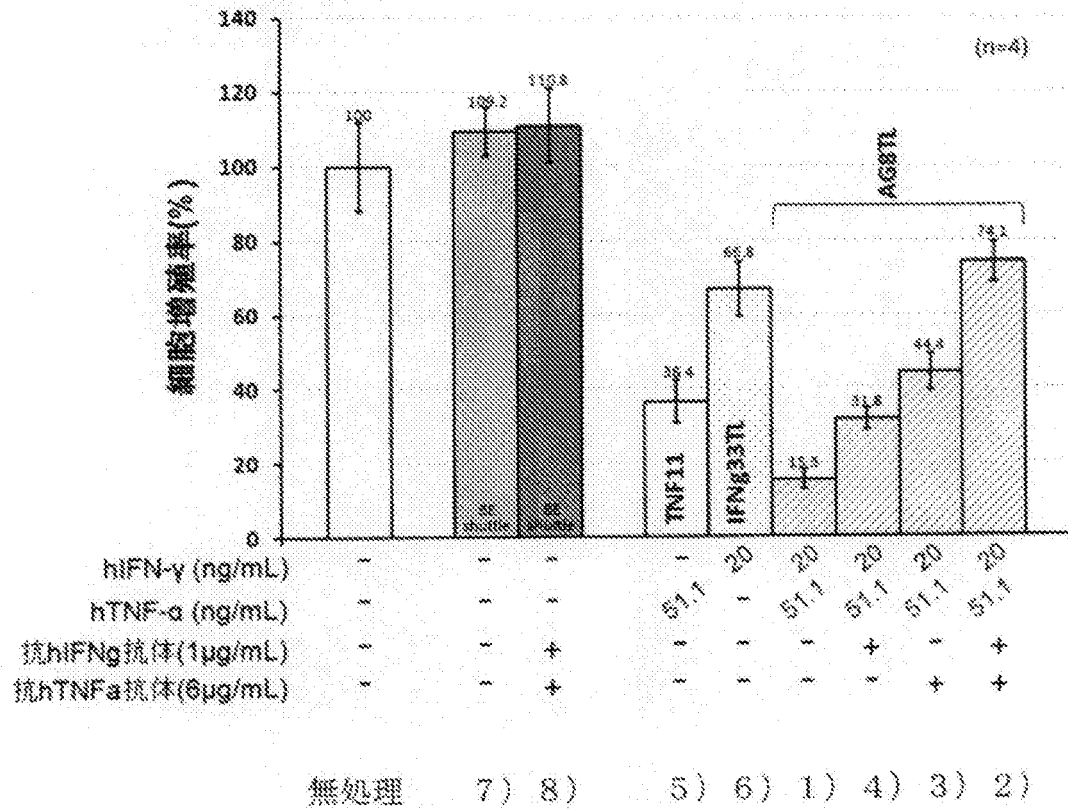
[図5]



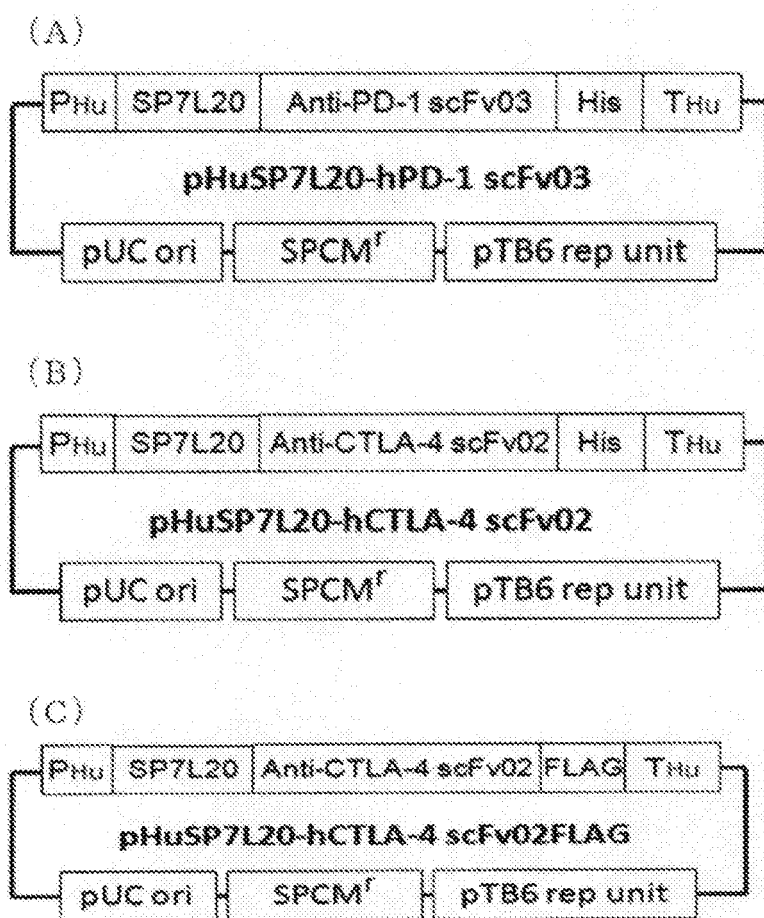
[図6]



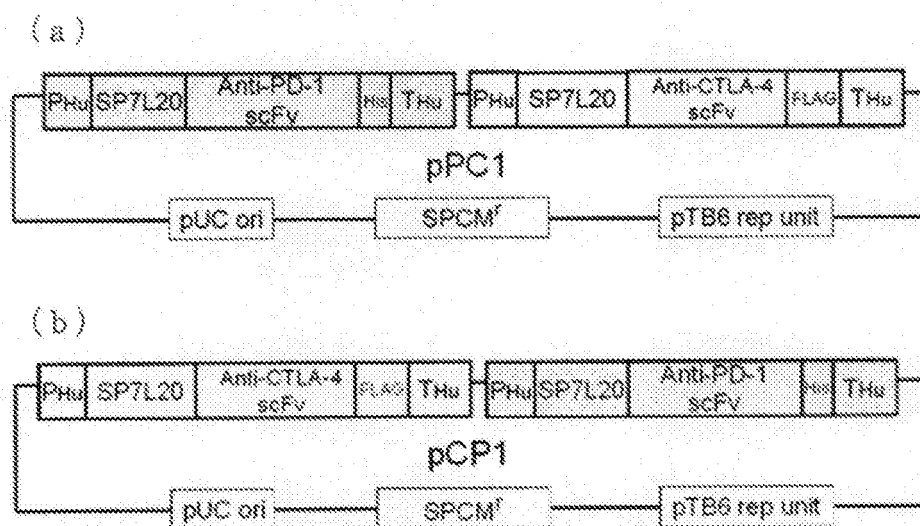
[図7]



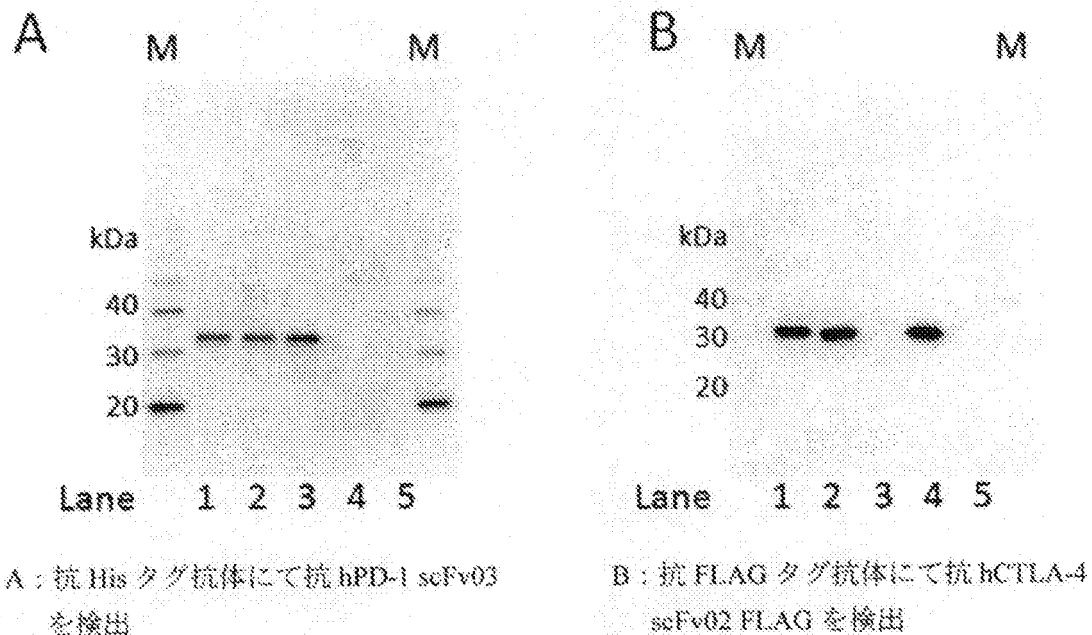
[図8]



[図9]



[図10]



レーン 1 : 共発現株 PC1 株 (抗 hPD-1 scFv03 の C 末端に His タグ融合, 抗 hCTLA-4 scFv02 の C 末端に FLAG タグ融合)

レーン 2 : 共発現株 CP1 株 (抗 hPD-1 scFv03 の C 末端に His タグ融合, 抗 hCTLA-4 scFv02 の C 末端に FLAG タグ融合)

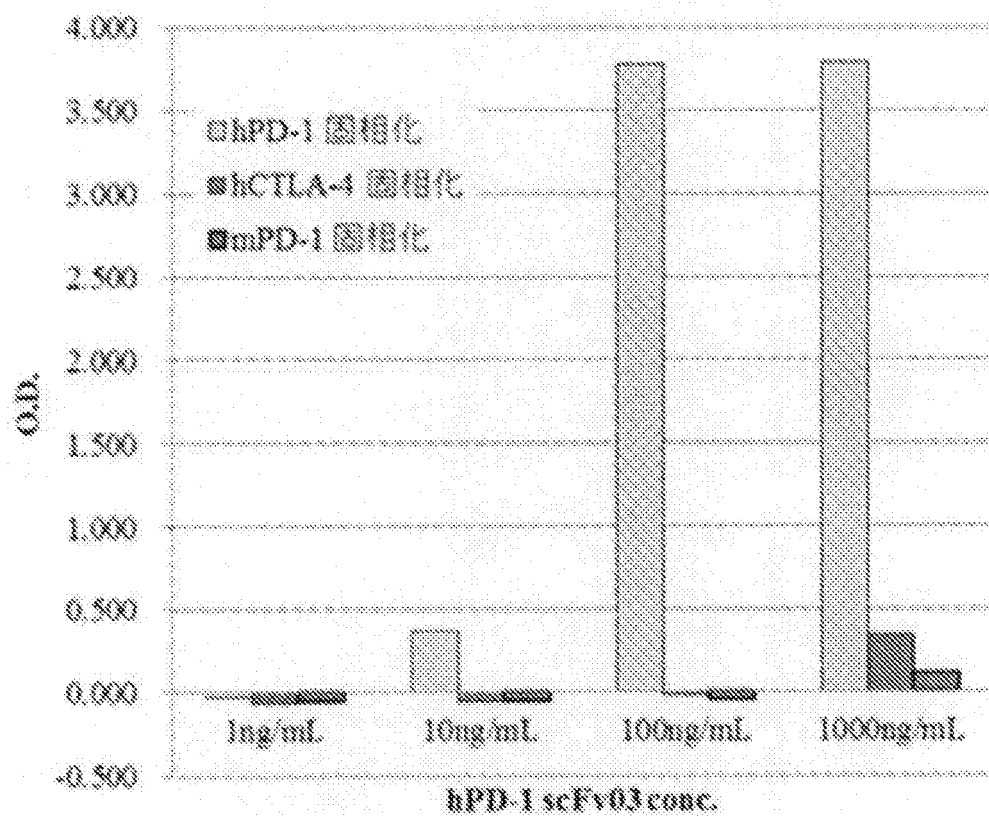
レーン 3 : 抗 hPD-1 scFv03 単独発現株 (抗 hPD-1 scFv03 の C 末端に His タグ融合)

レーン 4 : 抗 hCTLA-4 scFv02 単独発現株 (抗 hCTLA-4 scFv02 の C 末端に FLAG タグ融合)

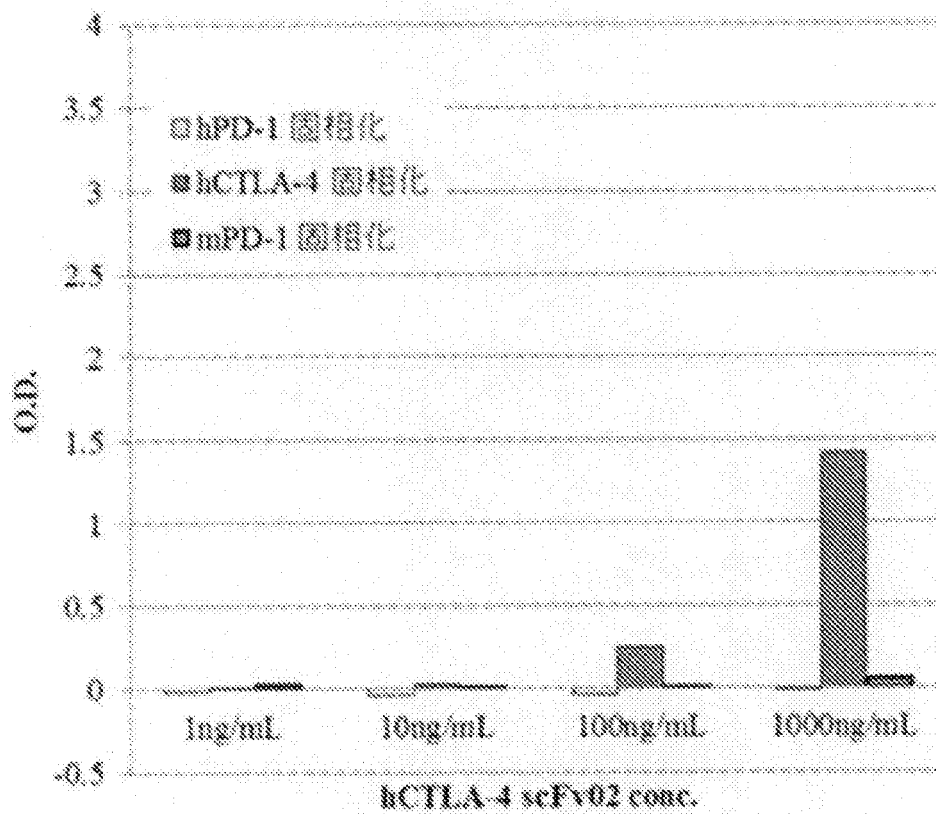
レーン 5 : 陰性対照株 Beshuttle 株

M : 分子量マーカー

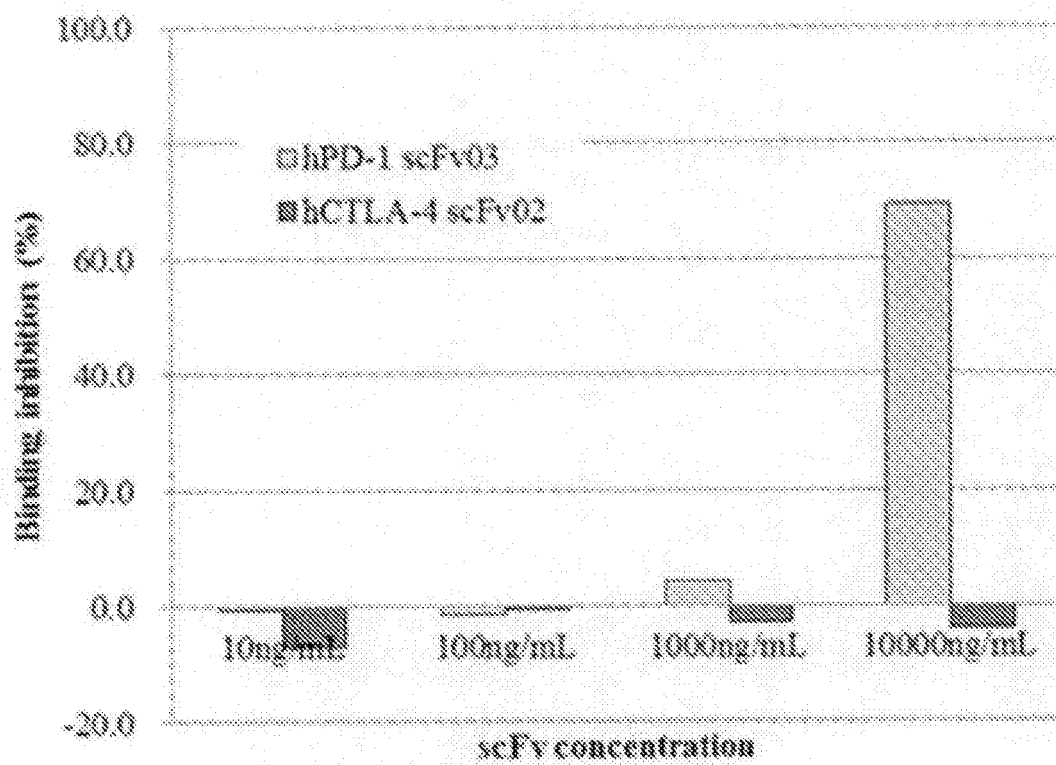
[図11]



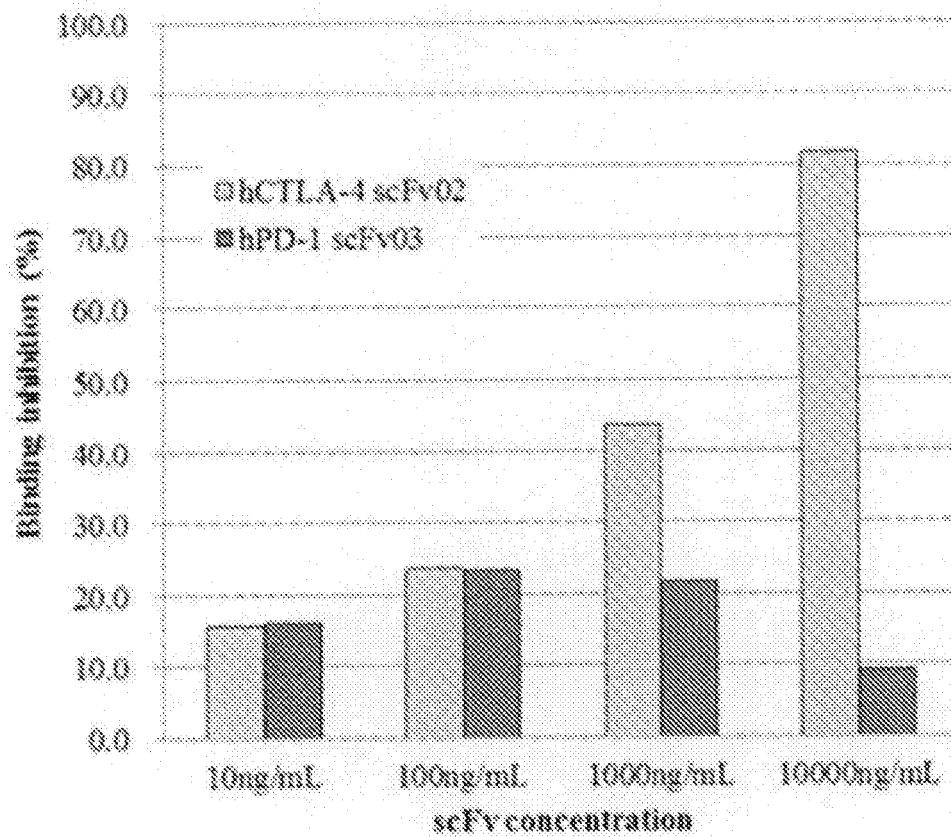
[図12]



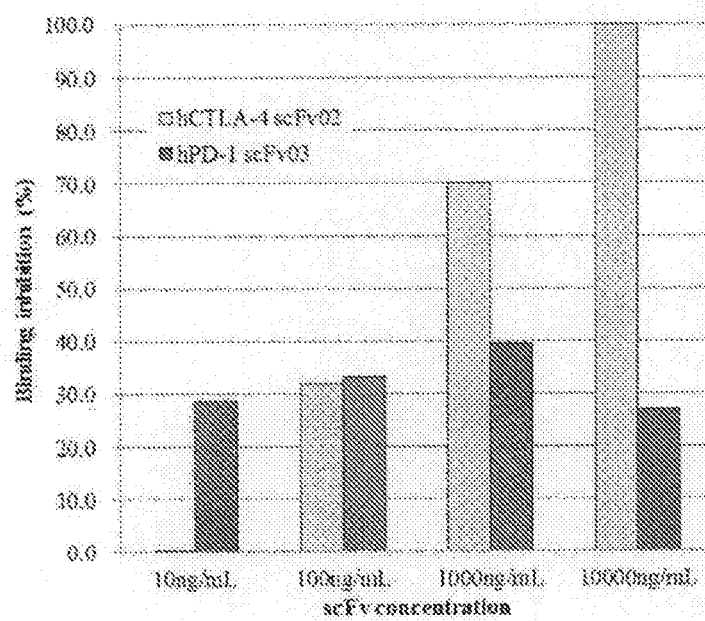
[図13]



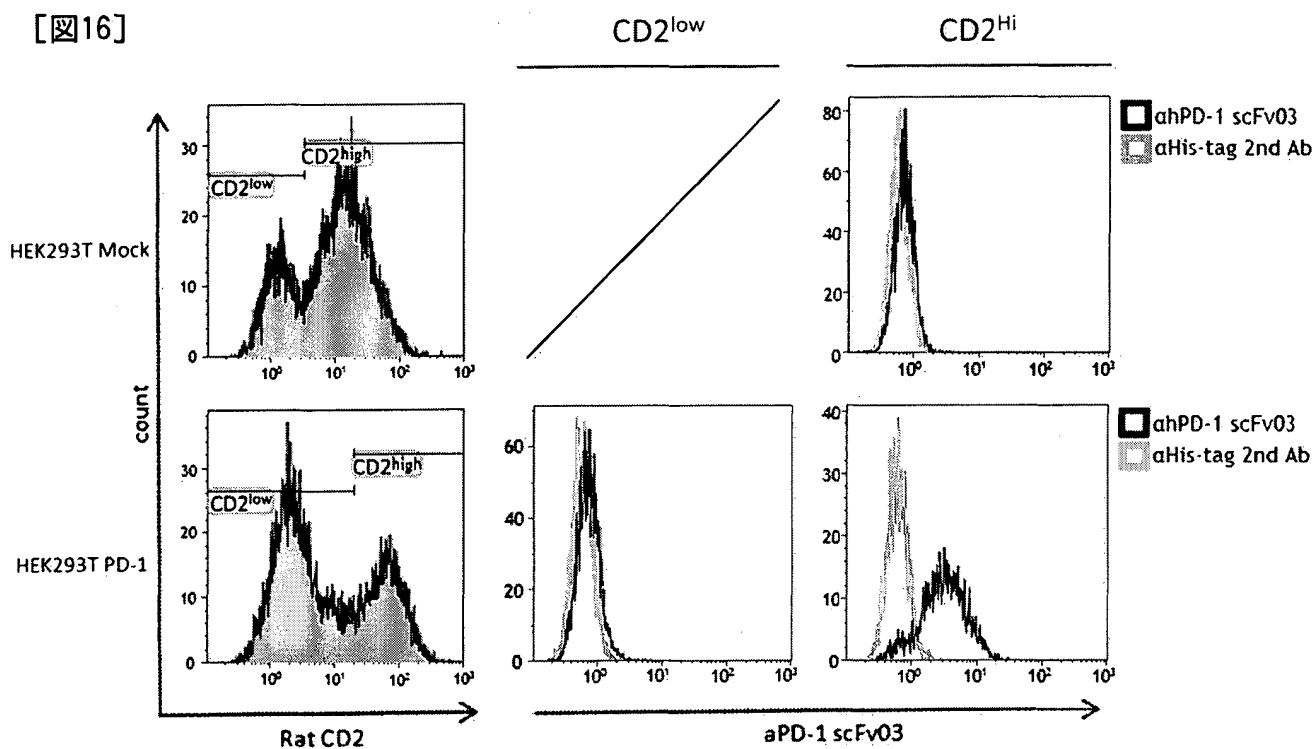
[図14]



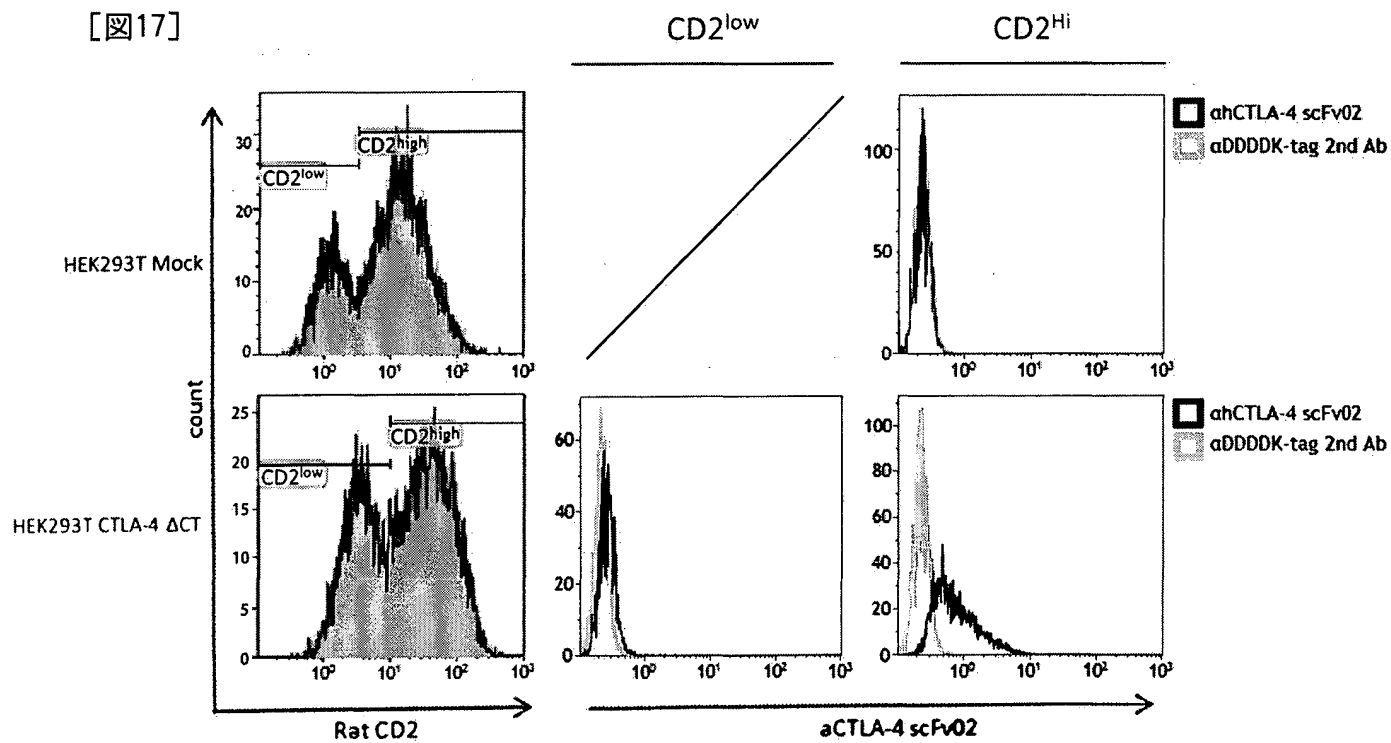
[図15]



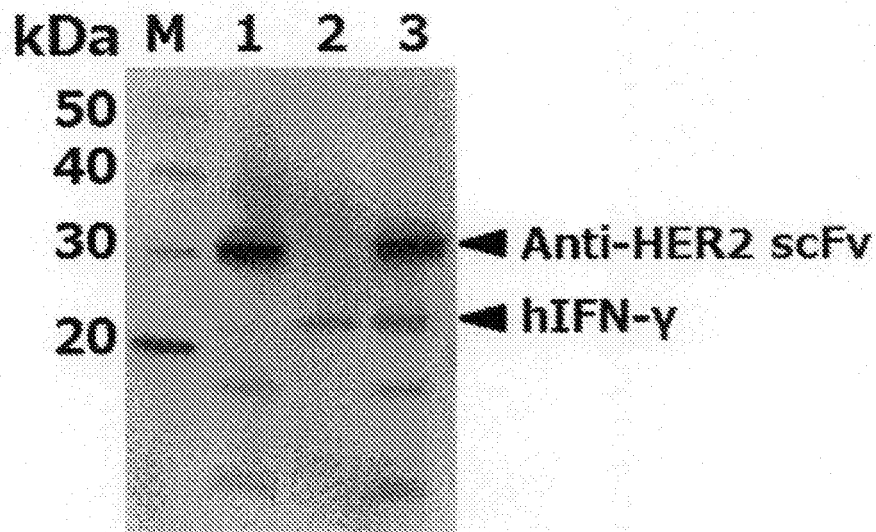
[図16]



[図17]



[図18]

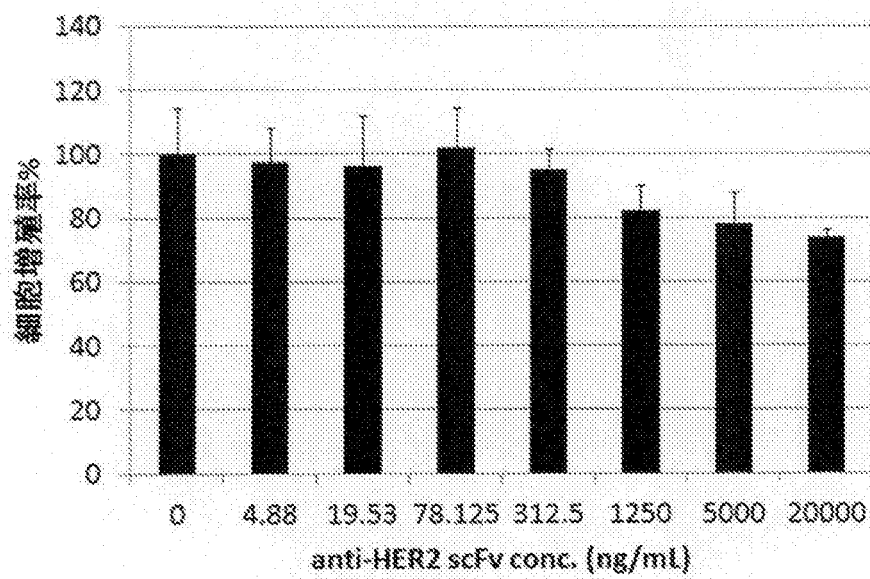


Lane 1: P30SP7L20-bHER2 株(抗 HER2 scFv 単独)

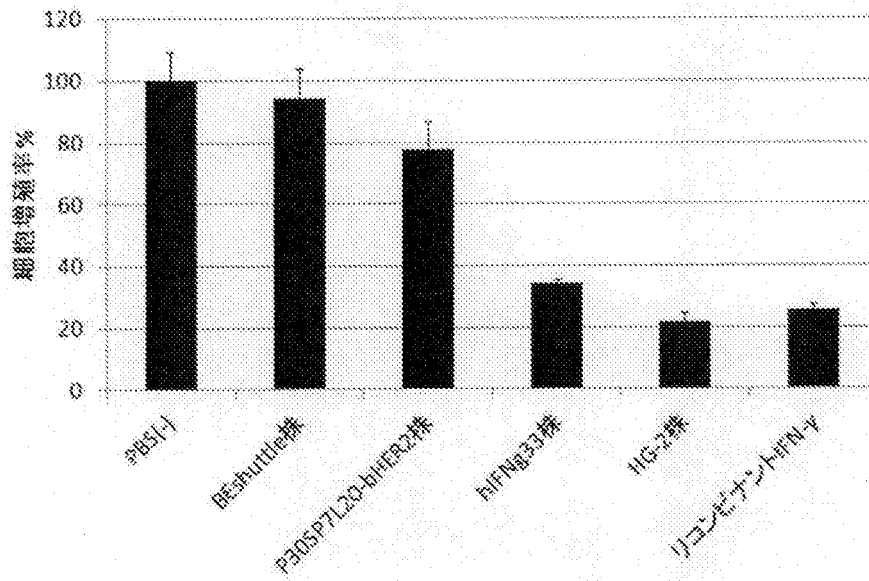
Lane 2: hIFNγ33 株(IFN-γ 単独)

Lane 3: HG-2 株(共発現)

[図19]

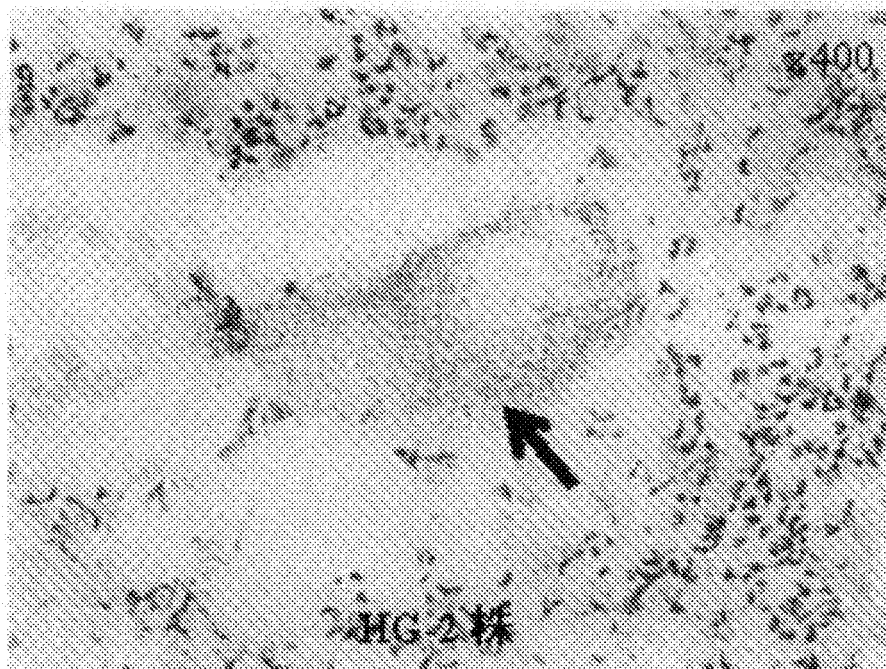


[図20]

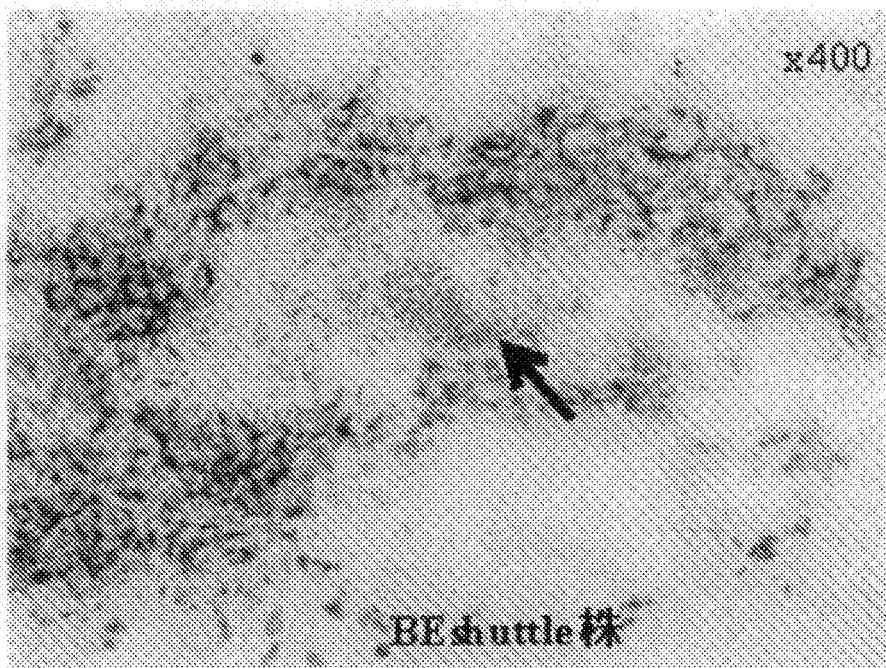


[図21]

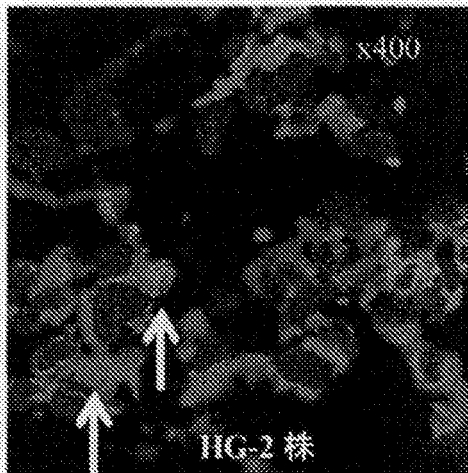
(A)



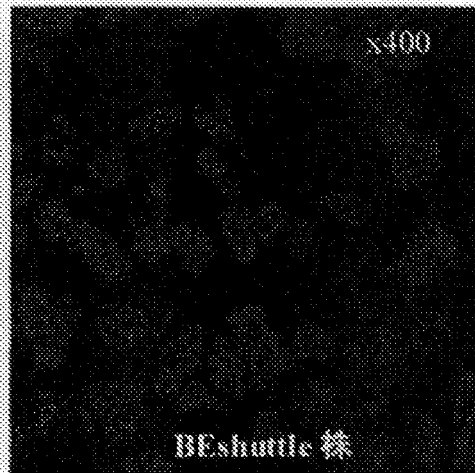
(B)



[図22]

抗 hIFN- γ 抗体染色 (↑ hIFN- γ : 緑)

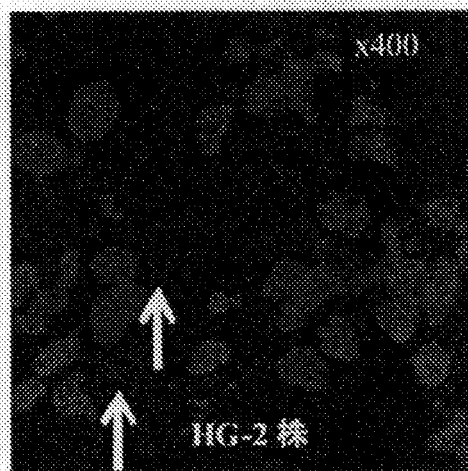
(A)



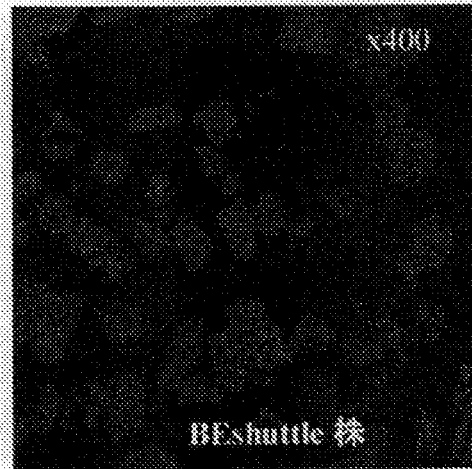
(B)

[図23]

抗ヒスチジントグ抗体染色 (↑ヒスチジントグ : 赤)

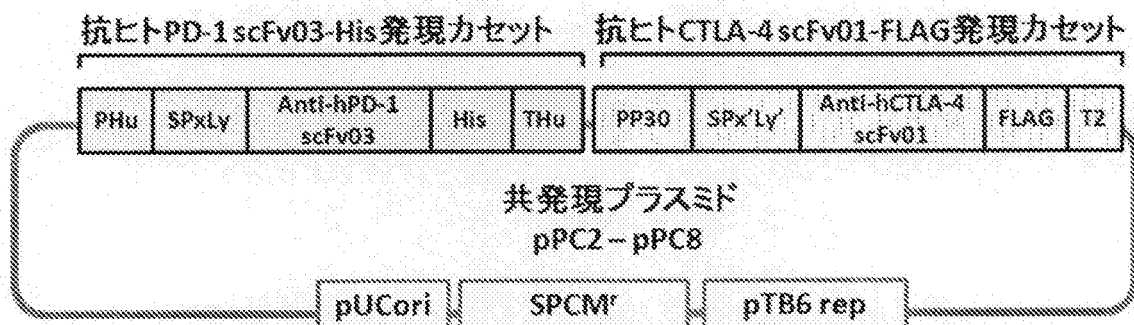


(A)

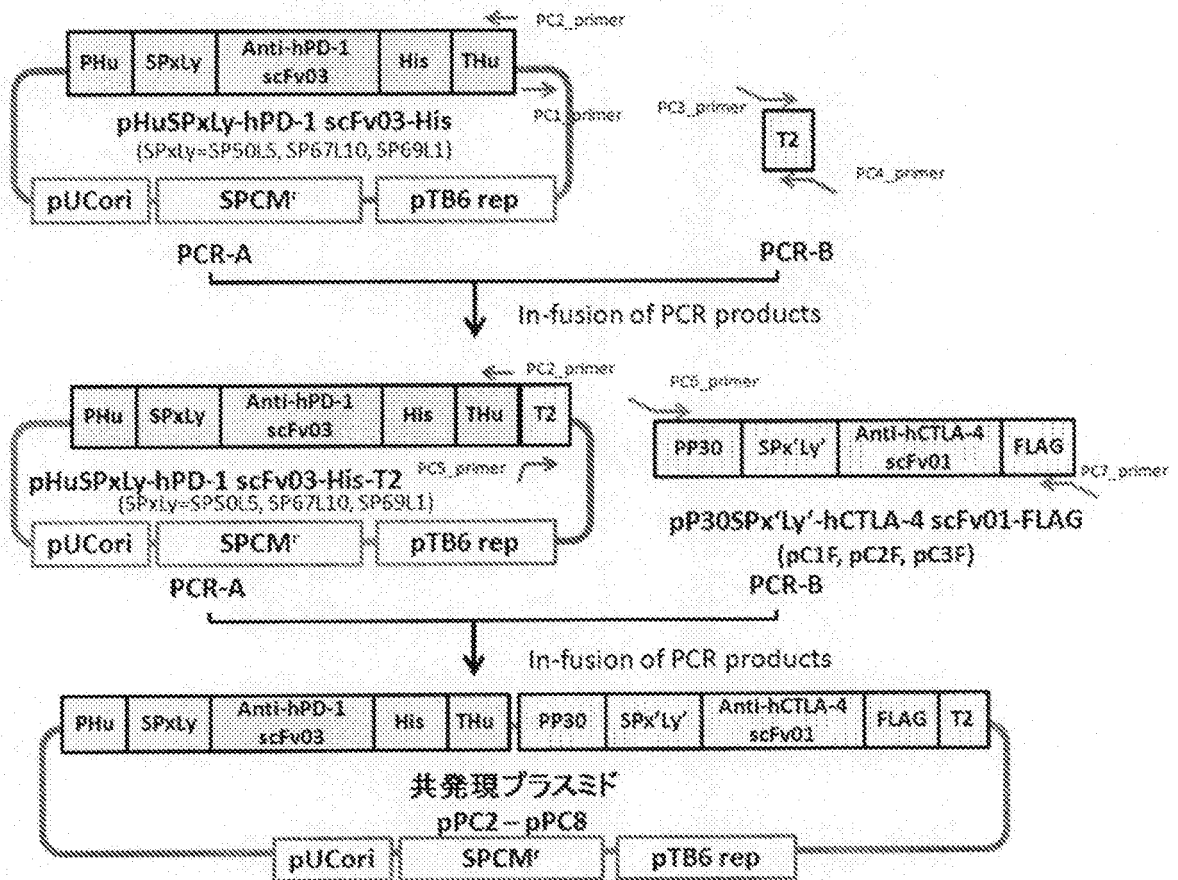


(B)

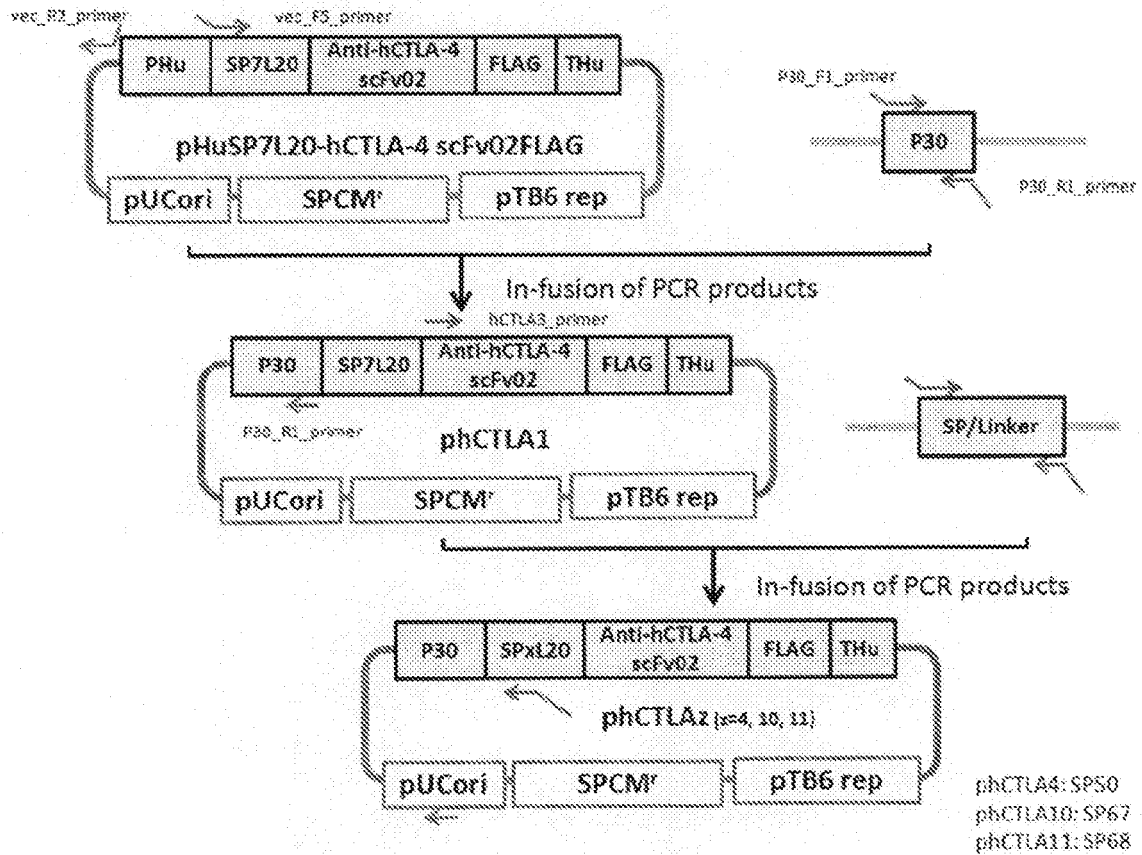
[図24]



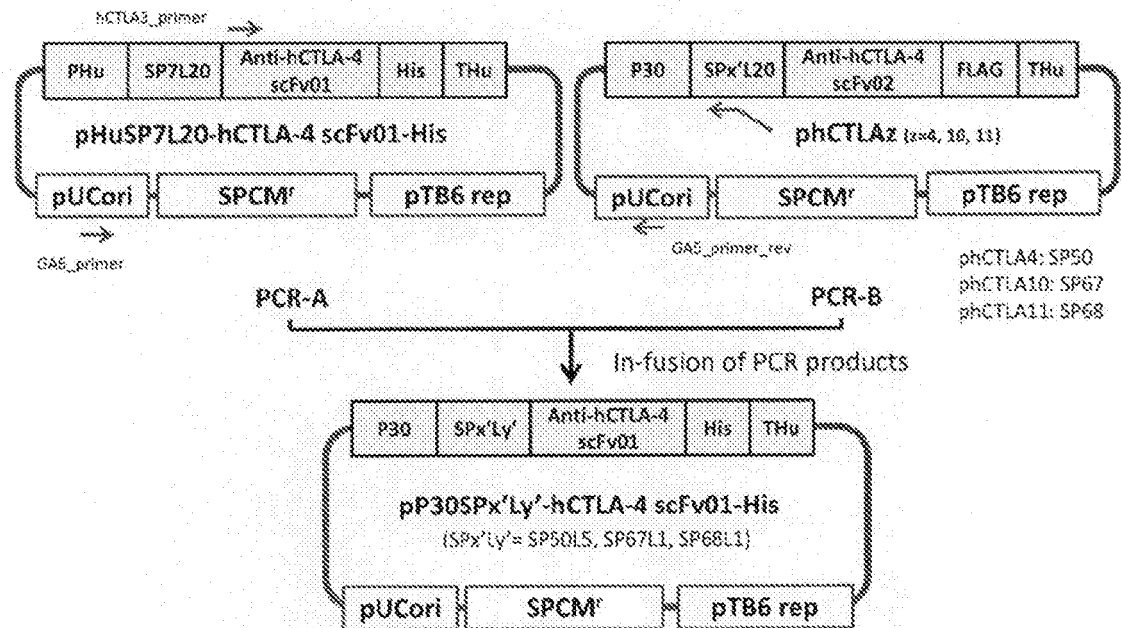
[図25]



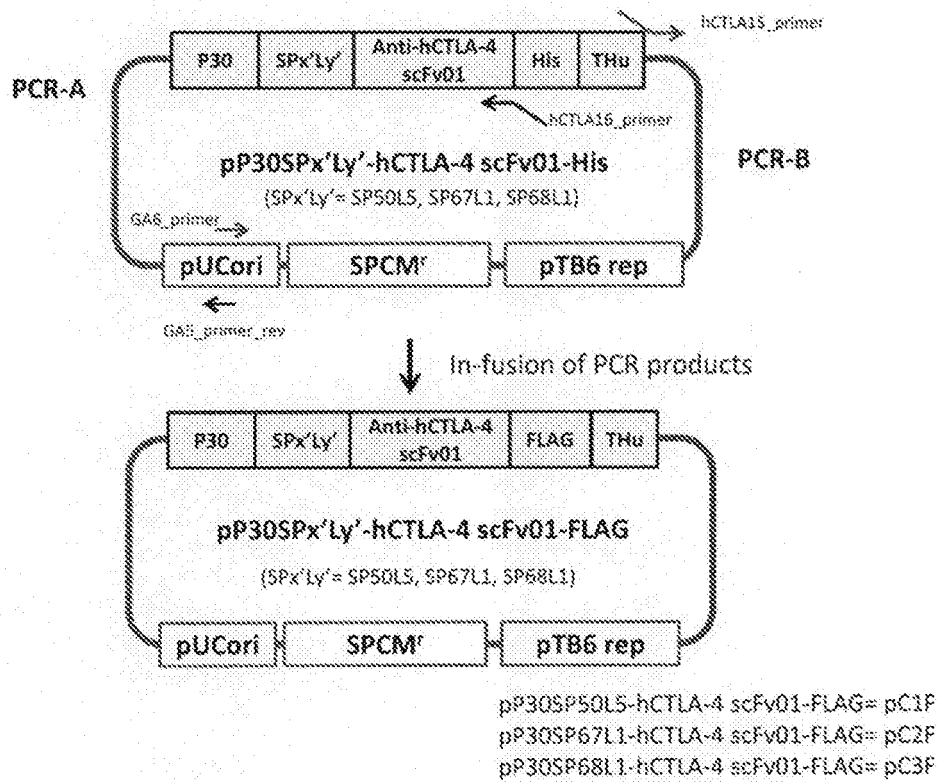
[図26]



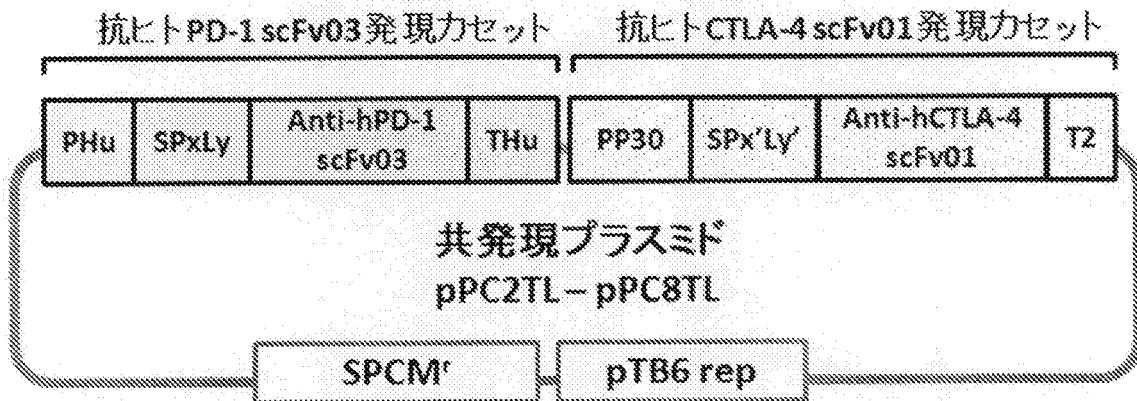
[図27]



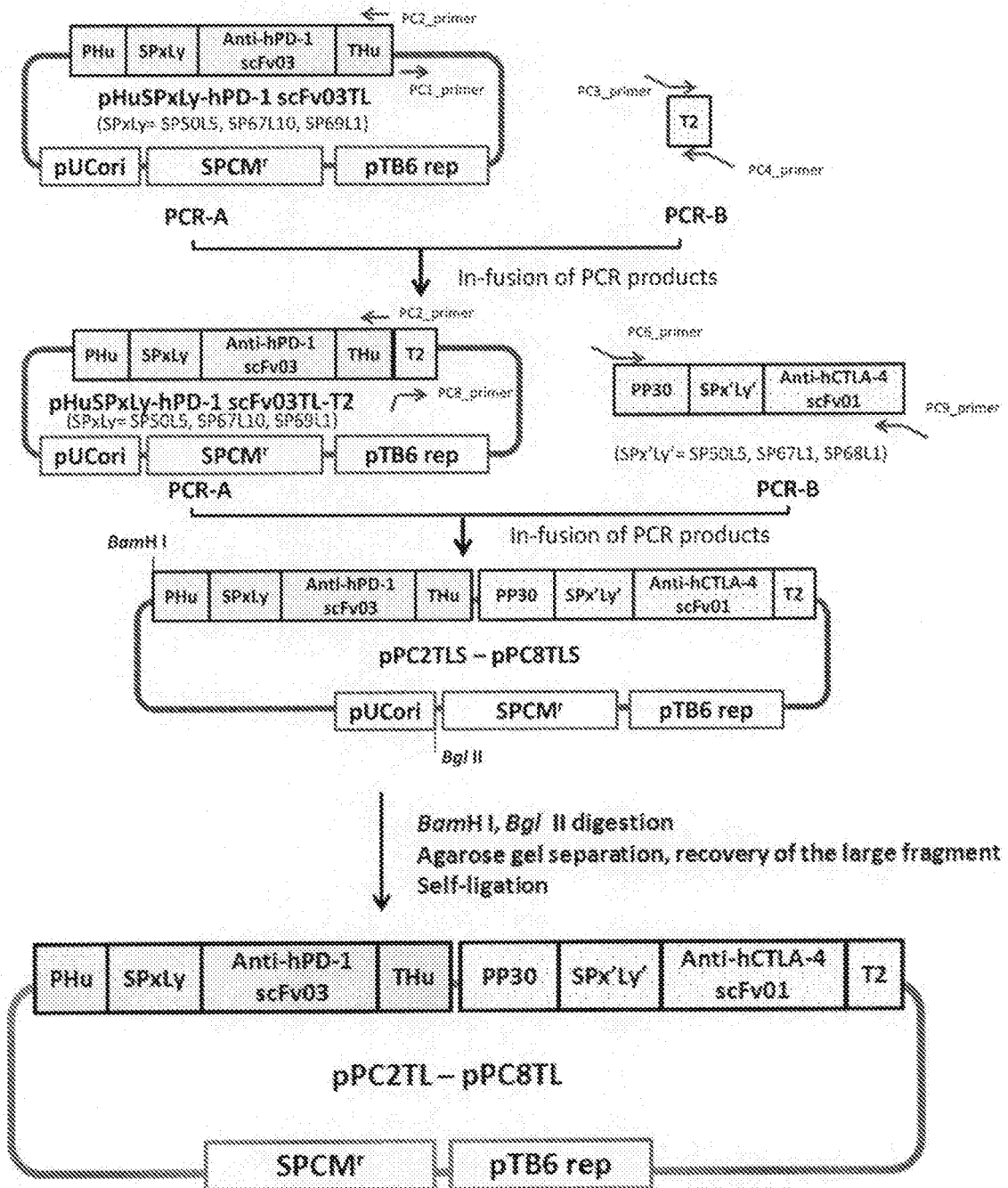
[図28]



[図29]



[図30]



1. PC2 (SP50L5-hPD-1-scFvO3-His, SP67L1-hCTLA4-scFvO1-FLAG)
2. PC3 (SP50L5-hPD-1-scFvO3-His, SP68L1-hCTLA4-scFvO1-FLAG)
3. PC4 (SP67L10-hPD-1-scFvO3-His, SP5P50L5-hCTLA4-scFvO1-FLAG)
4. PC5 (SP67L10-hPD-1-scFvO3-His, SP68L1-hCTLA4-scFvO1-FLAG)
5. PC6 (SP69L1-hPD-1-scFvO3-His, SP50L5-hCTLA4-scFvO1-FLAG)
6. PC7 (SP69L1-hPD-1-scFvO3-His, SP67L1-hCTLA4-scFvO1-FLAG)
7. PC8 (SP69L1-hPD-1-scFvO3-His, SP68L1-hCTLA4-scFvO1-FLAG)
8. Beshuttle (N.C.)
9. PC1

STEP 1

Bam HI

PP30 SPx'Ly' Anti-hCTLA-4 scFv01 FLAG THu

pC1F, pC2F, pC3F

PC1_primer

PC3_primer

T2: B8a-B0015 terminator

PCA_primer

pUCori SPCM' pTB6 rep

Bgl II

PCR-A

PCR-B

In-fusion of PCR products

Bam HI

PP30 SPx'Ly' Anti-hCTLA-4 scFv01 T2

pC1TL, pC2TL, pC3TL

Bgl II

pUCori SPCM' pTB6 rep

- *Bam* HI, *Bgl* II digestion
- Separation by agarose gel electrophoresis
- Recover the large fragment (4 kbp)
- Self-ligation

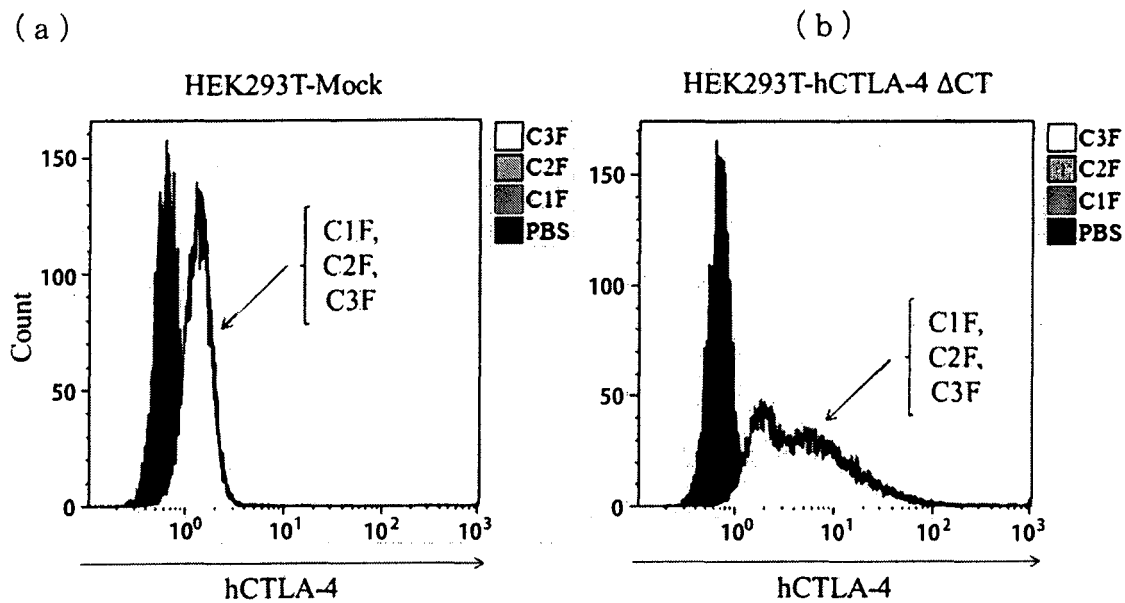
STEP 2

PP30 SPx'Ly' Anti-hCTLA-4 scFv01 T2

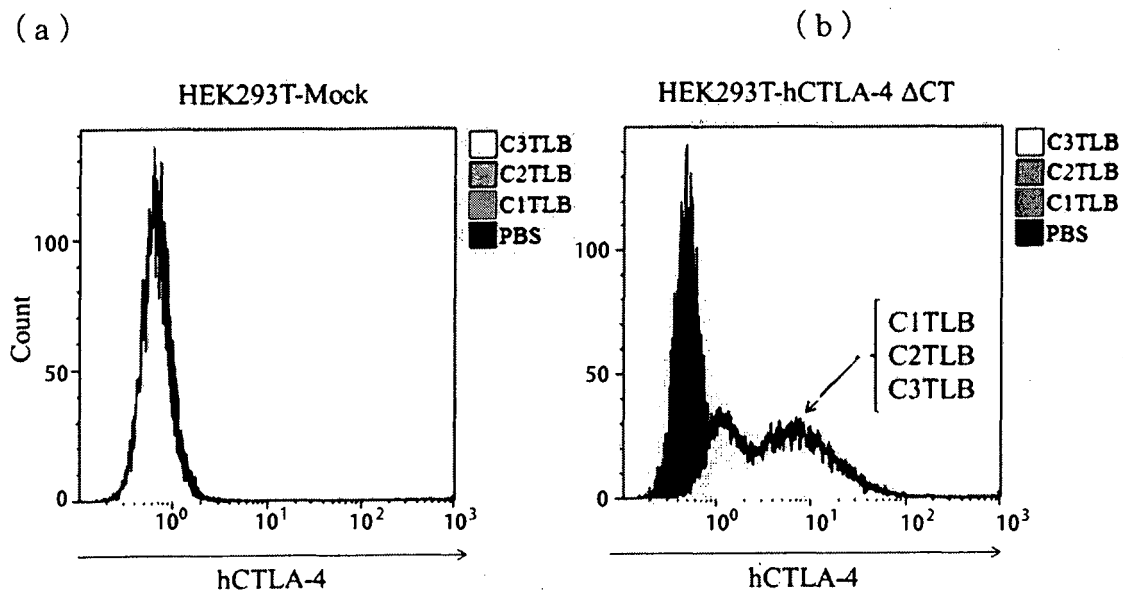
pC1TLB, pC2TLB, pC3TLB

SPCM' pTB6 rep

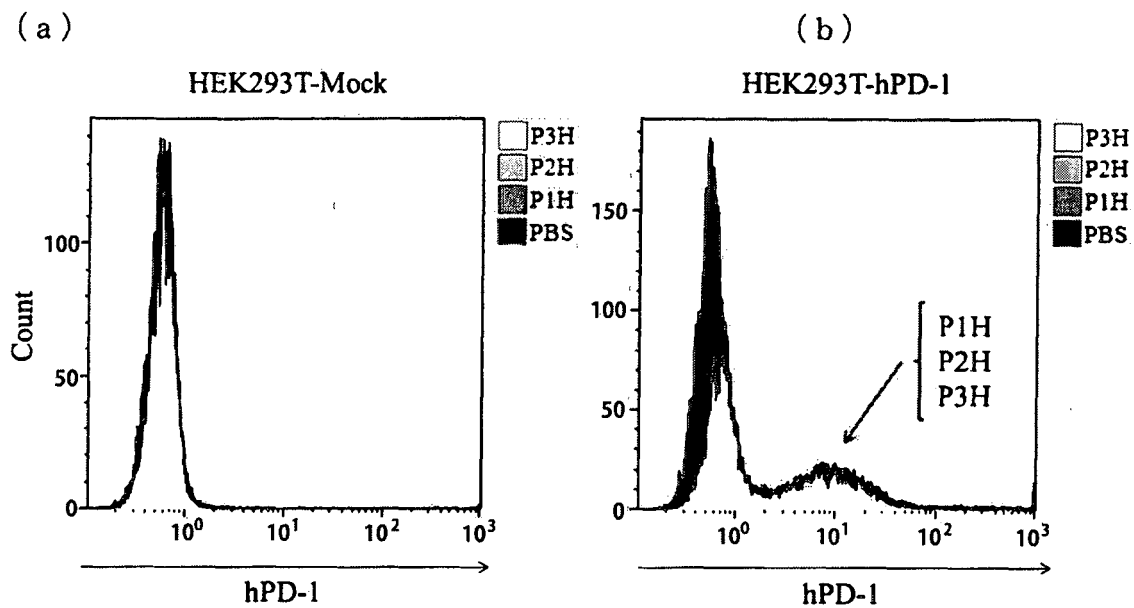
[図33]



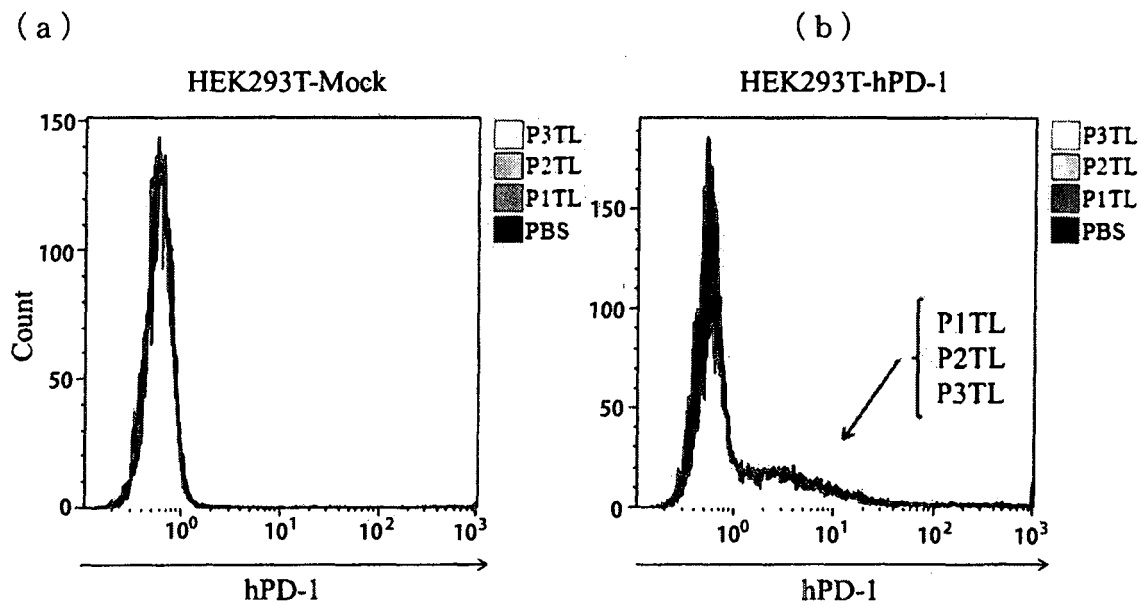
[図34]



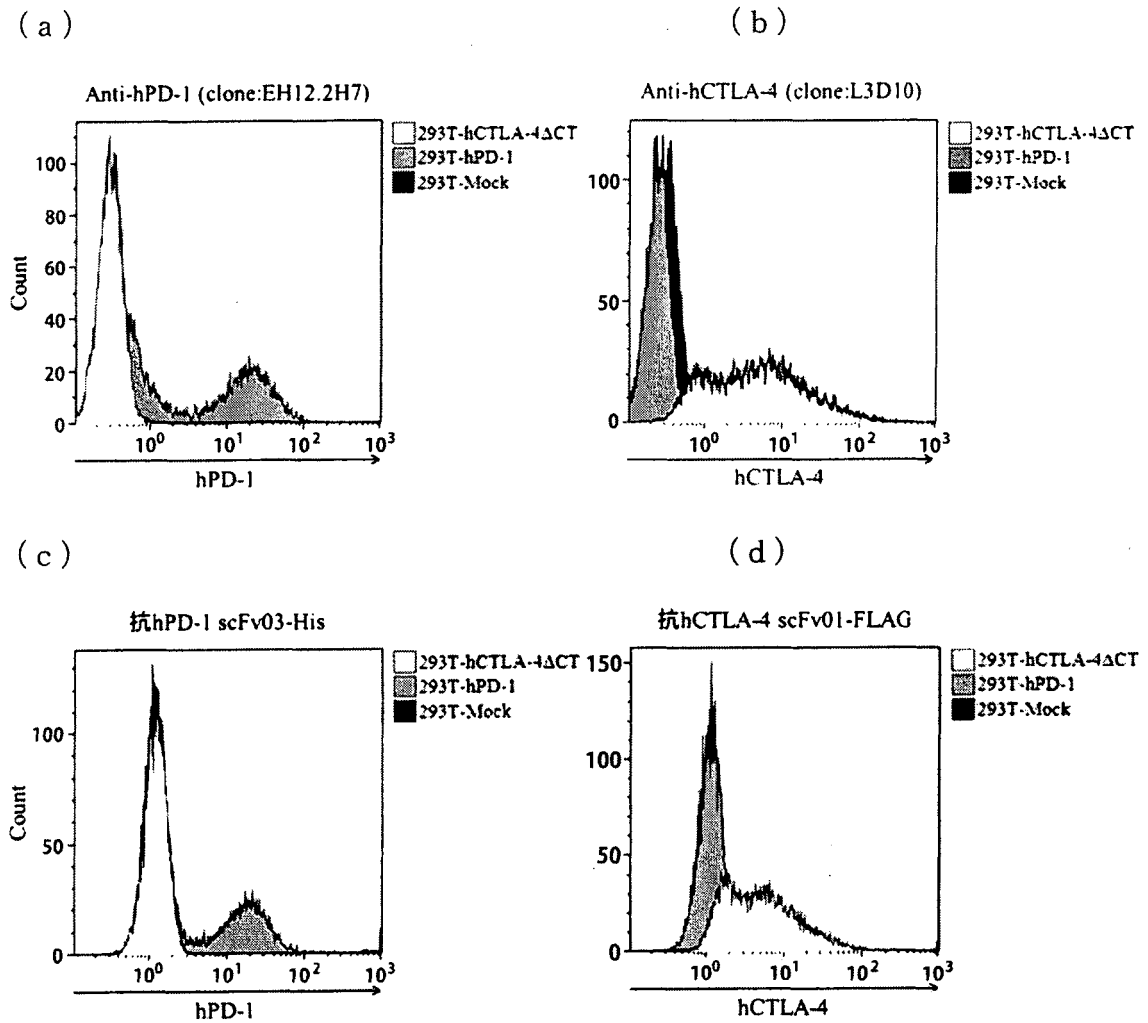
[図35]



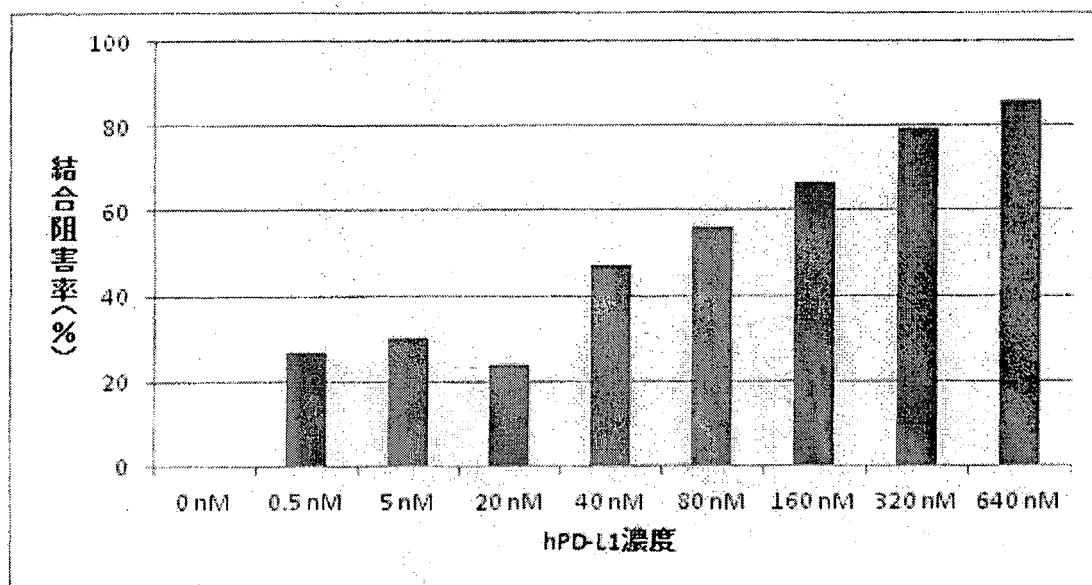
[図36]



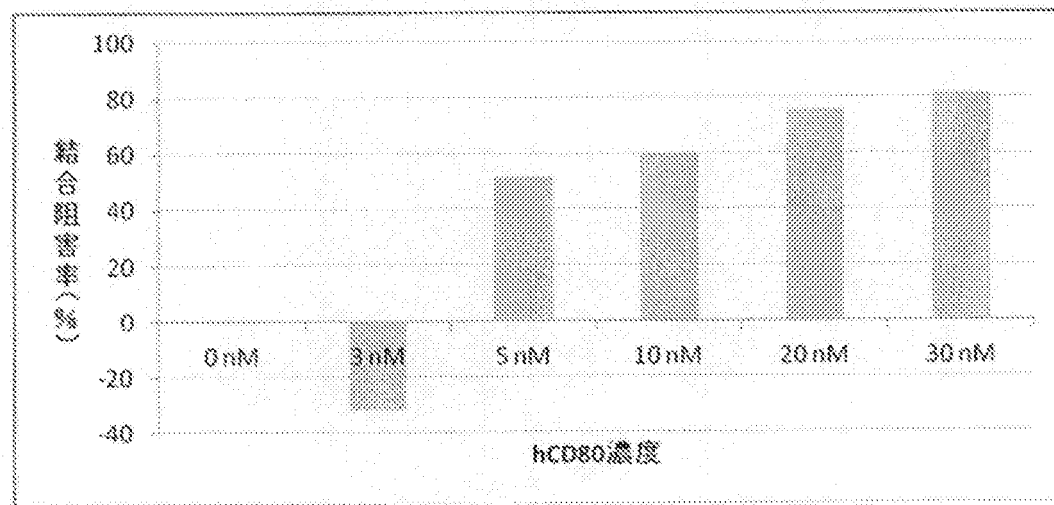
[図37]



[図38]



[図39]



[図40]

