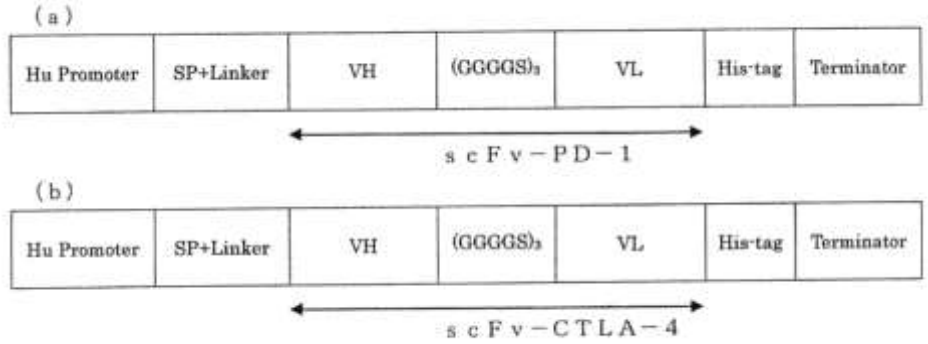
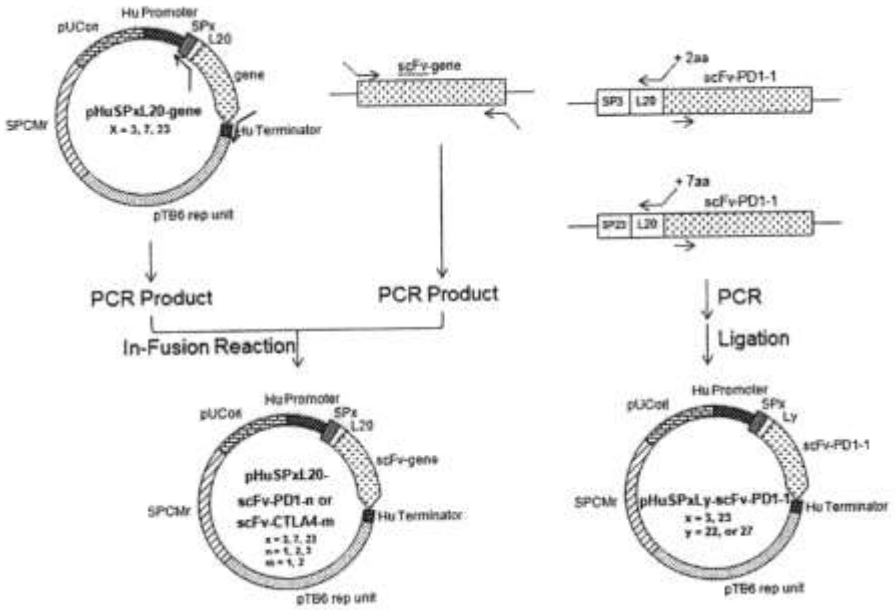
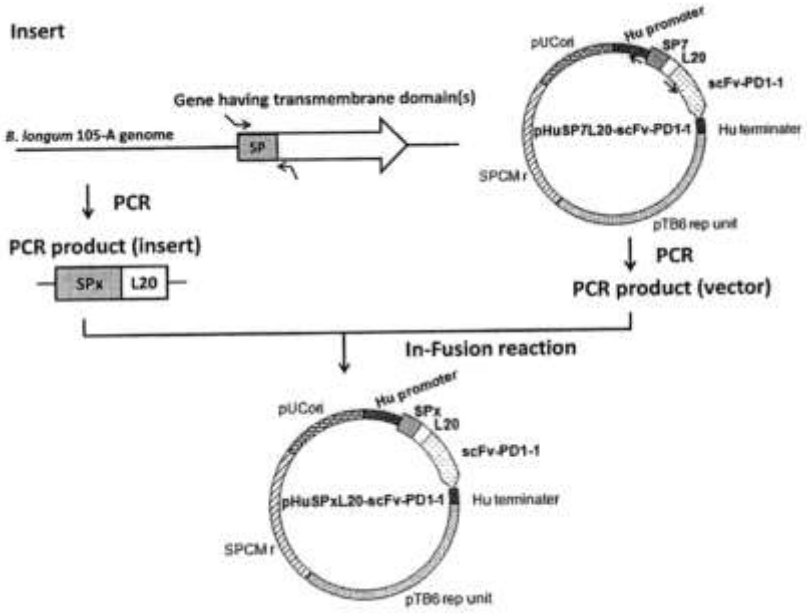
[Figure 1]



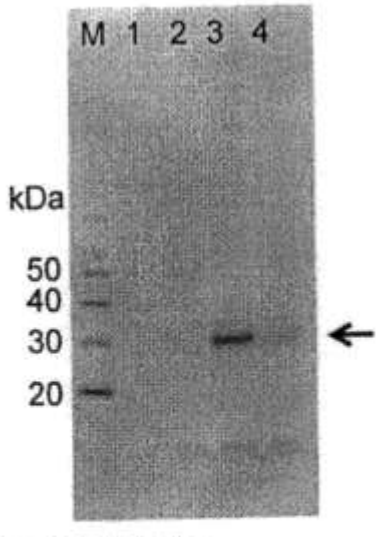
[Figure 2]



[Figure 3]

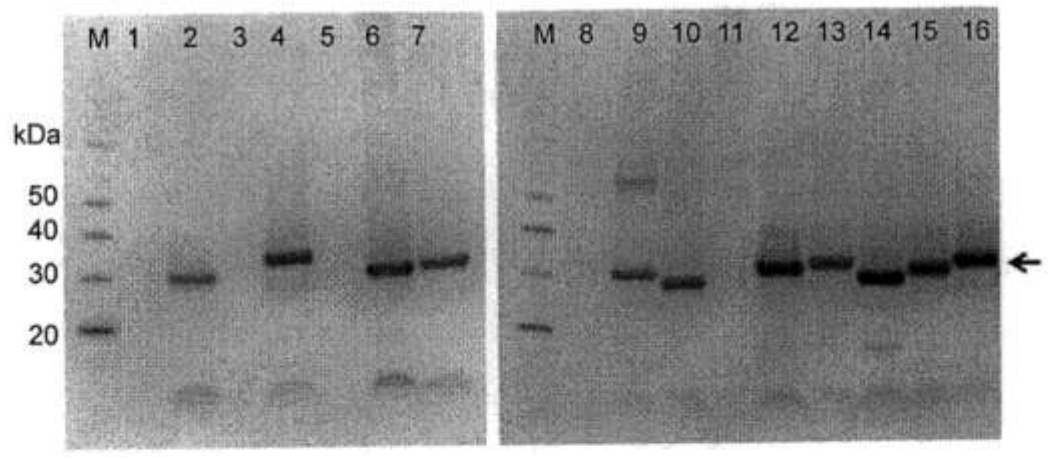


[Figure 4]



Lane M: Marker
Lane 1: Negative control
Lane 2: *Bifidobacterium longum* 105-A/pHuSP3L22-scFv-PD-1-1 strain
Lane 3: *Bifidobacterium longum* 105-A/pHuSP7L20-scFv-PD-1-1 strain
Lane 4: *Bifidobacterium longum* 105-A/pHuSP23L27-scFv-PD-1-1 strain

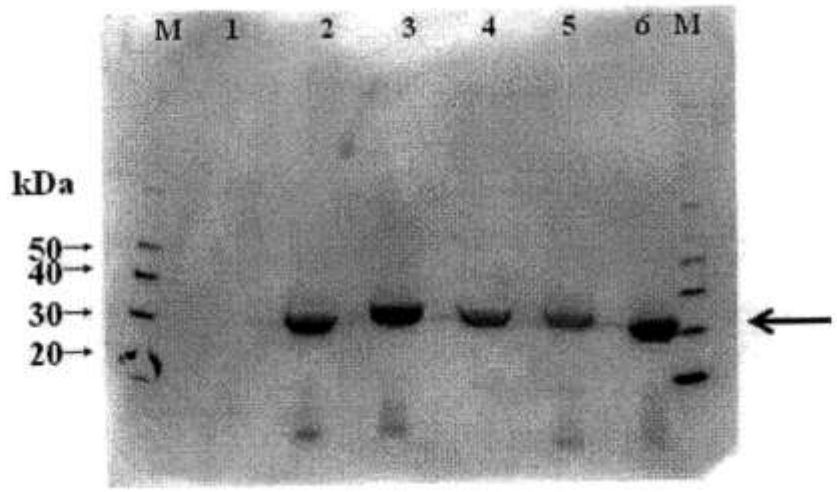
[Figure 5]



Signal peptide-linker peptide conjugate used in *Bifidobacterium longum* 105-A/pHuSPxL20-scFv-PD-1-1 strain

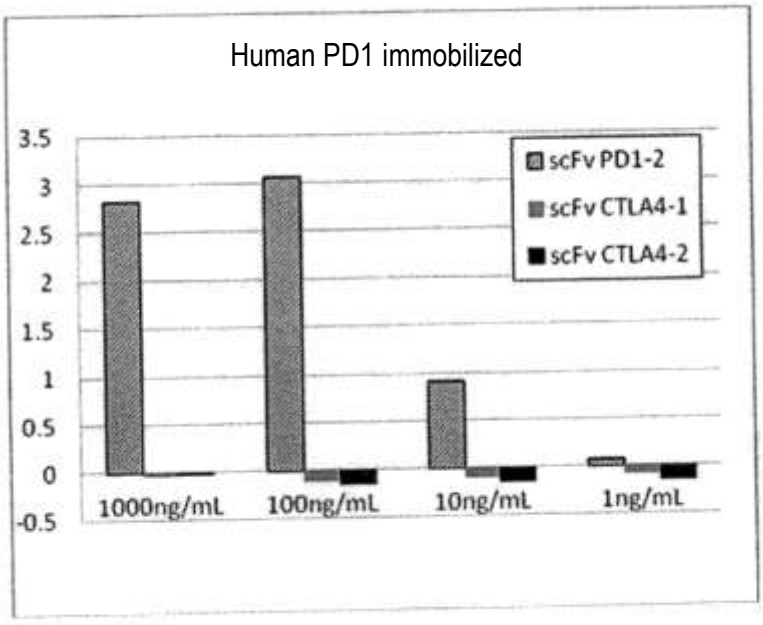
Lane 1, 8: Negative control (pBEshuttle)
Lane 2: SP7L20
Lane 3: SP42L20
Lane 4: SP45L20
Lane 5: SP48L20
Lane 6: SP50L20
Lane 7: SP52L20
Lane 9: SP55L20
Lane 10: SP58L20
Lane 11: SP62L20
Lane 12: SP64L20
Lane 13: SP66L20
Lane 14: SP67L20
Lane 15: SP68L20
Lane 16: SP69L20

[Figure 6]

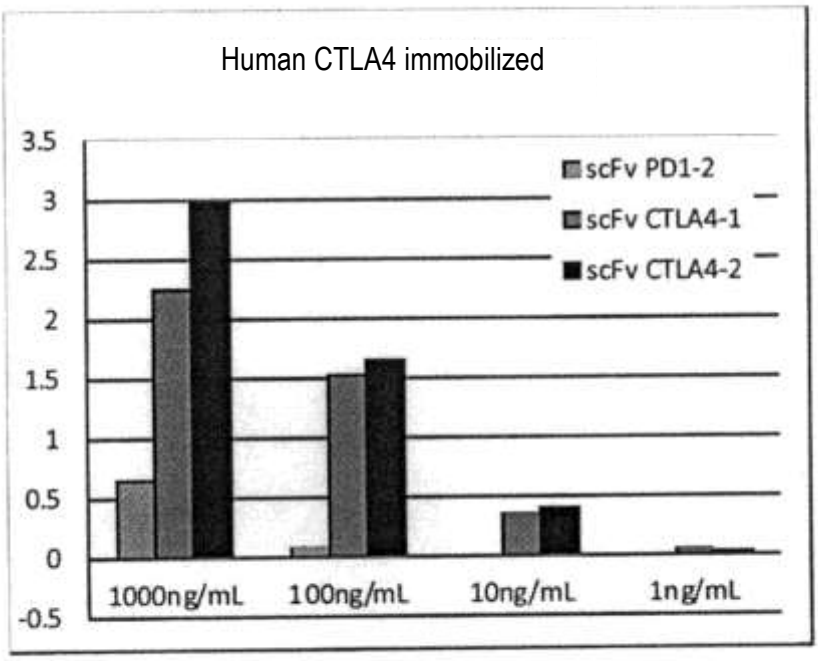


Lane 1: Negative control (pBEshuttle strain)
Lane 2: *Bifidobacterium longum* 105-A/pHuSP7L20-PD-1-1 strain
Lane 3: *Bifidobacterium longum* 105-A/pHuSP7L20-PD-1-2 strain
Lane 4: *Bifidobacterium longum* 105-A/pHuSP7L20-PD-1-3 strain
Lane 5: *Bifidobacterium longum* 105-A/pHuSP7L20-CTLA-4-1 strain
Lane 6: *Bifidobacterium longum* 105-A/pHuSP7L20-CTLA-4-2 strain
M: Molecular-weight Marker

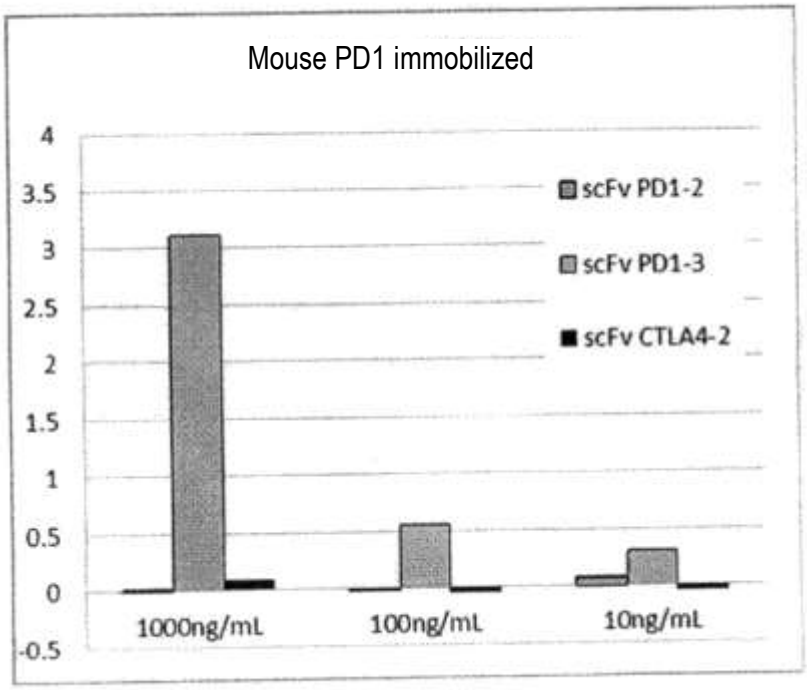
[Figure 7]



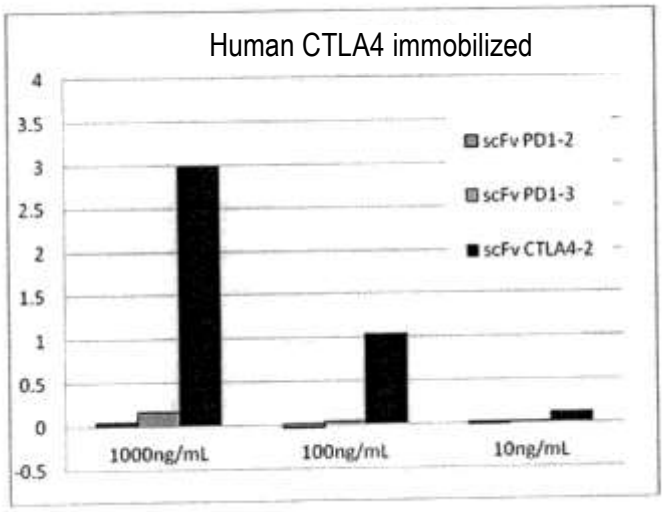
[Figure 8]



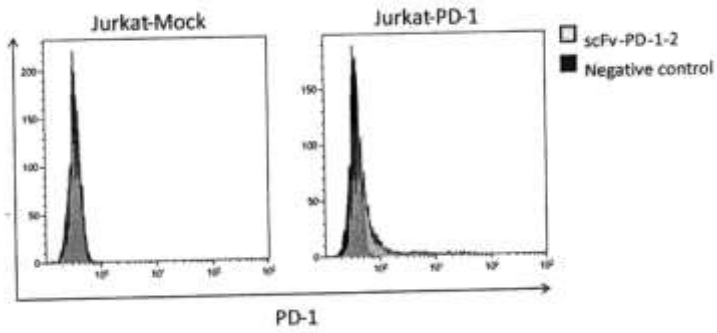
[Figure 9]



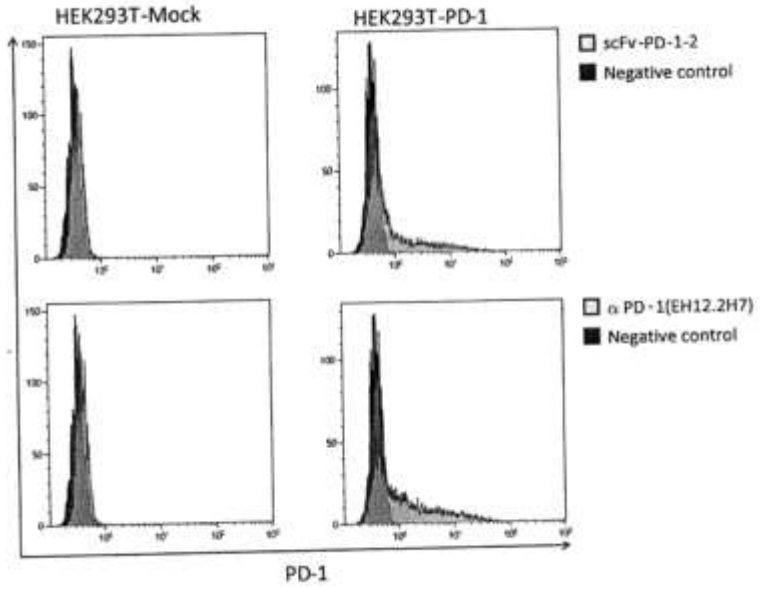
[Figure 10]



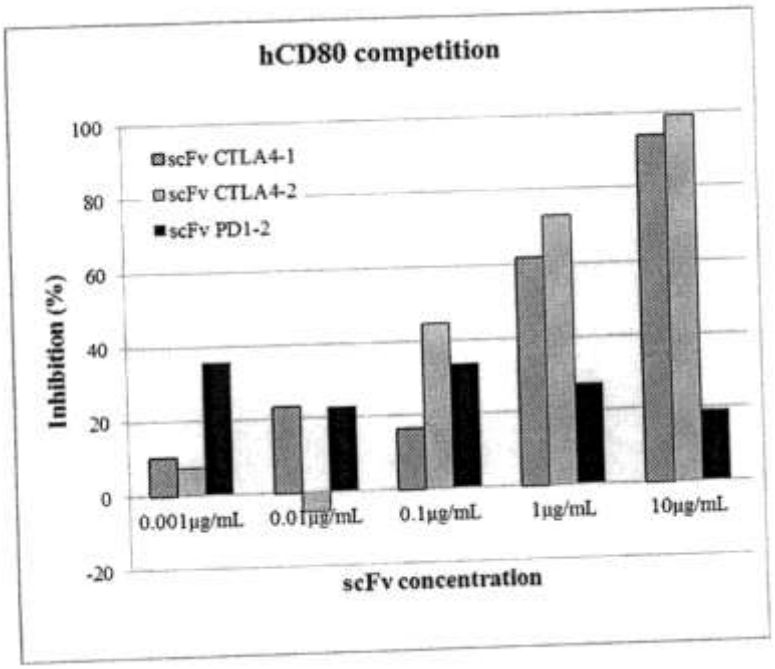
[Figure 11]



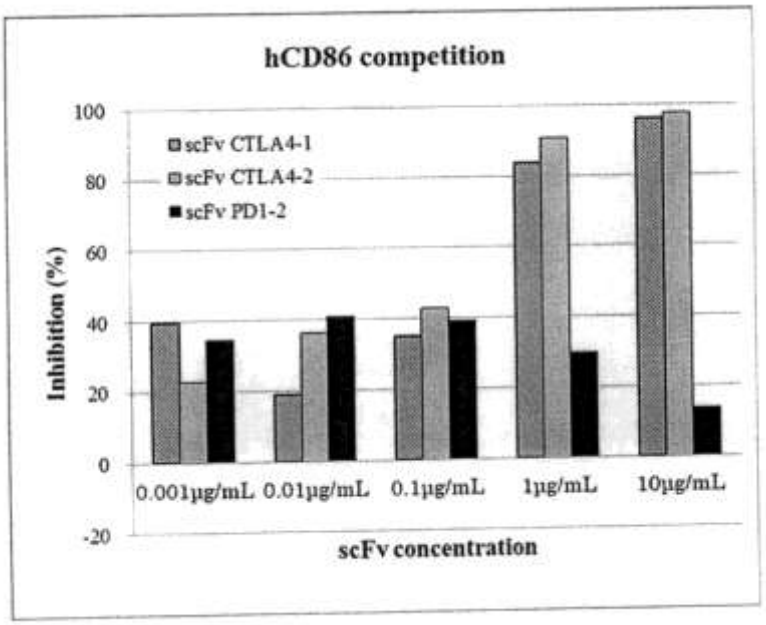
[Figure 12]



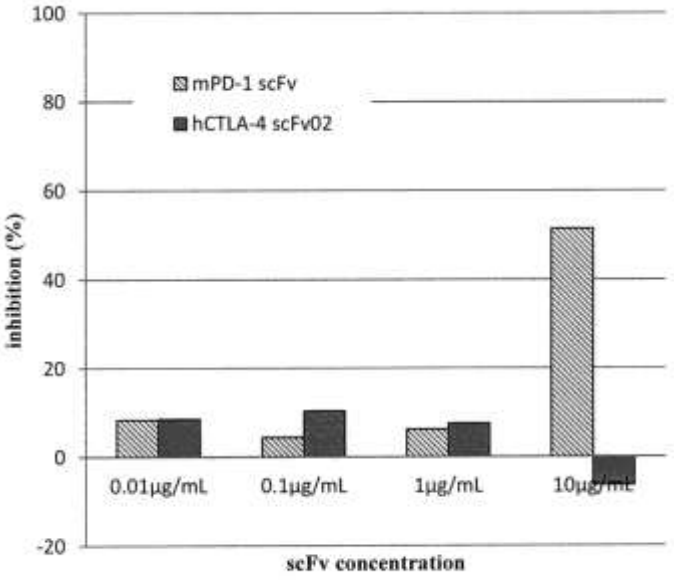
[Figure 13]



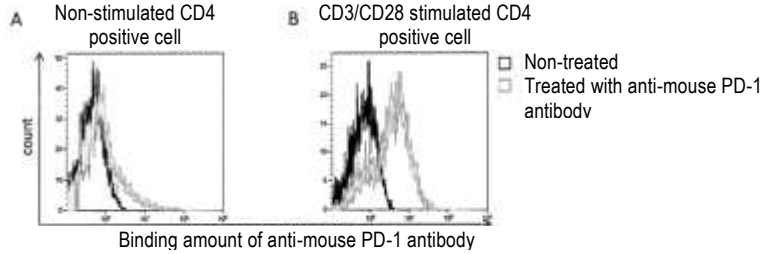
[Figure 14]



[Figure 15]



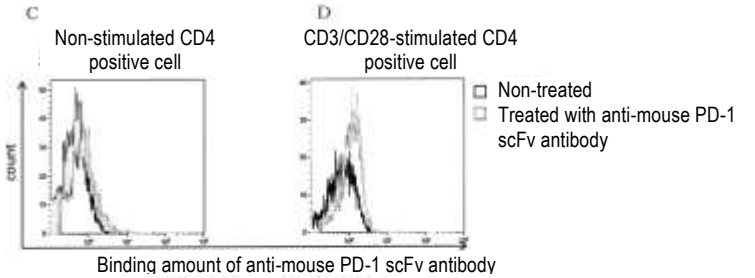
[Figure 16]



Induction of expression of mPD-1 by stimulation of CD4 positive T cell with CD3/CD28 (analysis by flow cytometry)

A. Anti-mouse PD-1 antibody (□) was reacted with non-stimulated CD4 positive cell and detection was made by FITC

B. Anti-mouse PD-1 antibody (□) was reacted with CD3/CD28-stimulated CD4 positive cell and detection was made by FITC

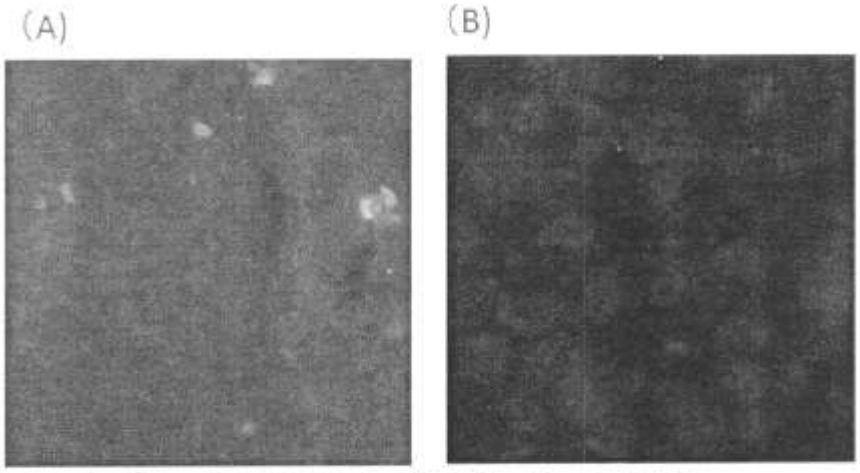


Binding of anti-mouse PD-1 scFv antibody (□) to CD3 and CD28 stimulated CD4 positive cells (analysis by flow cytometry)

C. Anti-mouse PD-1 scFv antibody (□) was reacted with non-stimulated CD4 positive cell and Alexa488·anti-His tag antibody was reacted with His thereof and detection was made

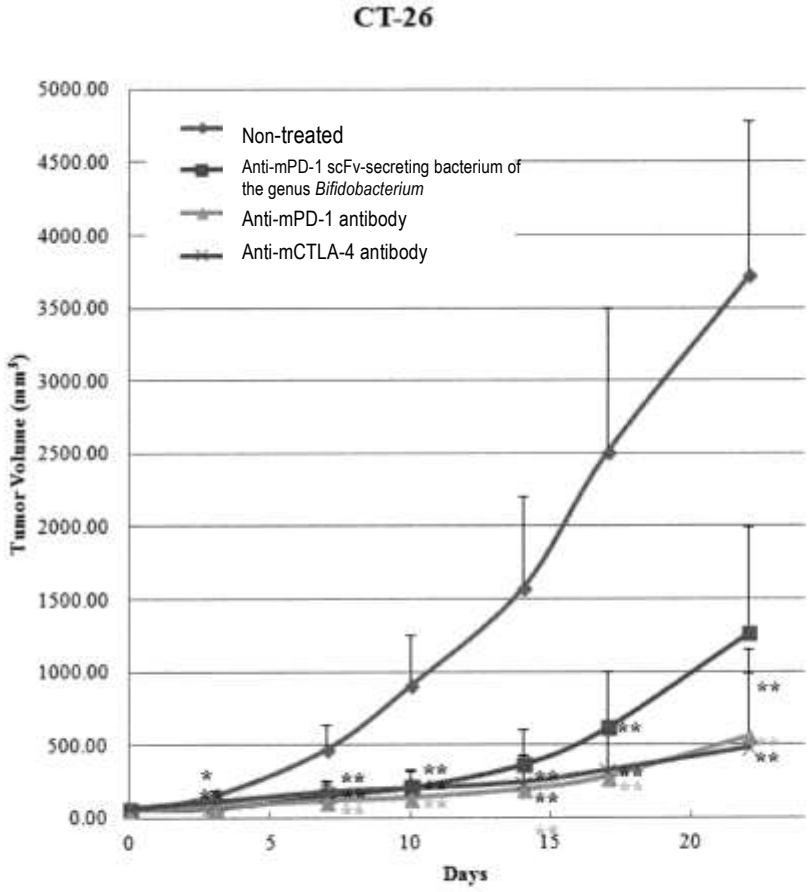
D. Anti-mouse PD-1 scFv antibody was reacted with CD3 and CD28 stimulated CD4 positive cell and Alexa488·anti-His tag antibody was reacted with His thereof and detection was made

[Figure 17]



A. Tumor tissue of an anti-mouse PD-1 scFv antibody-secreting bacterium of the genus *Bifidobacterium* administered mouse
B. Tumor tissue of not administered mouse

[Figure 18]

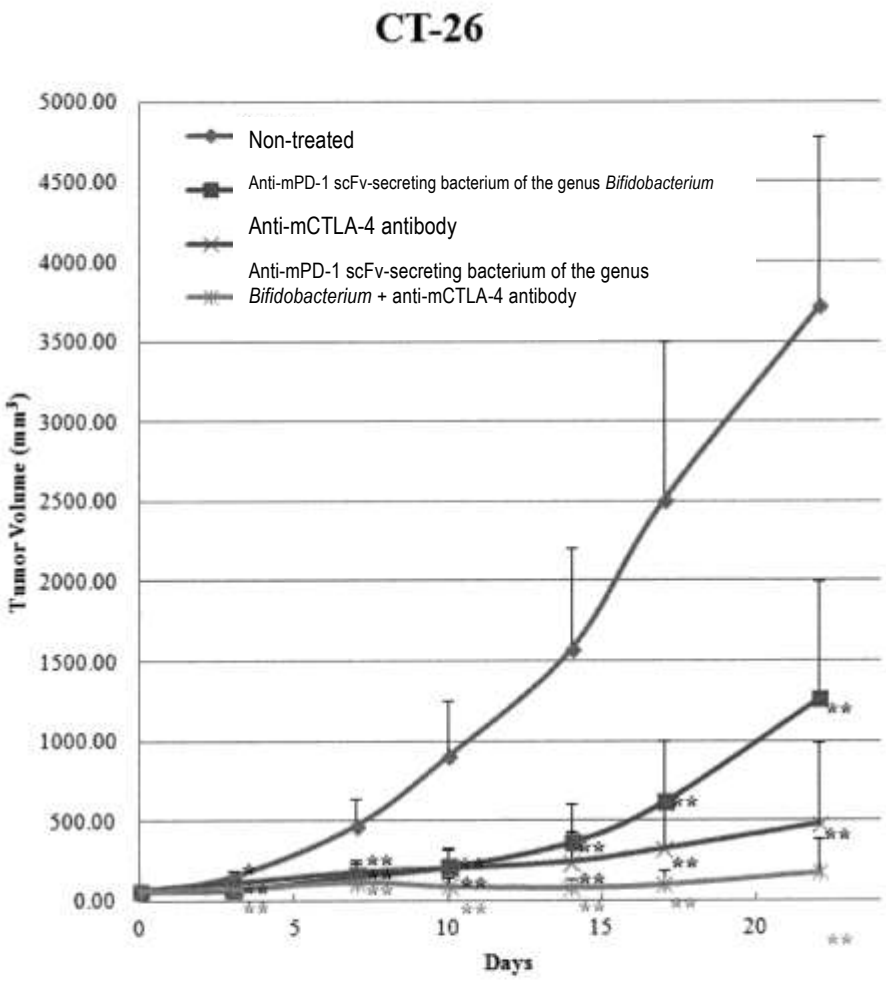


The mean values + S.D. of seven mice.

*: P value < 0.05 (vs Non-treated)

**: P value < 0.01 (vs Non-treated)

[Figure 19]

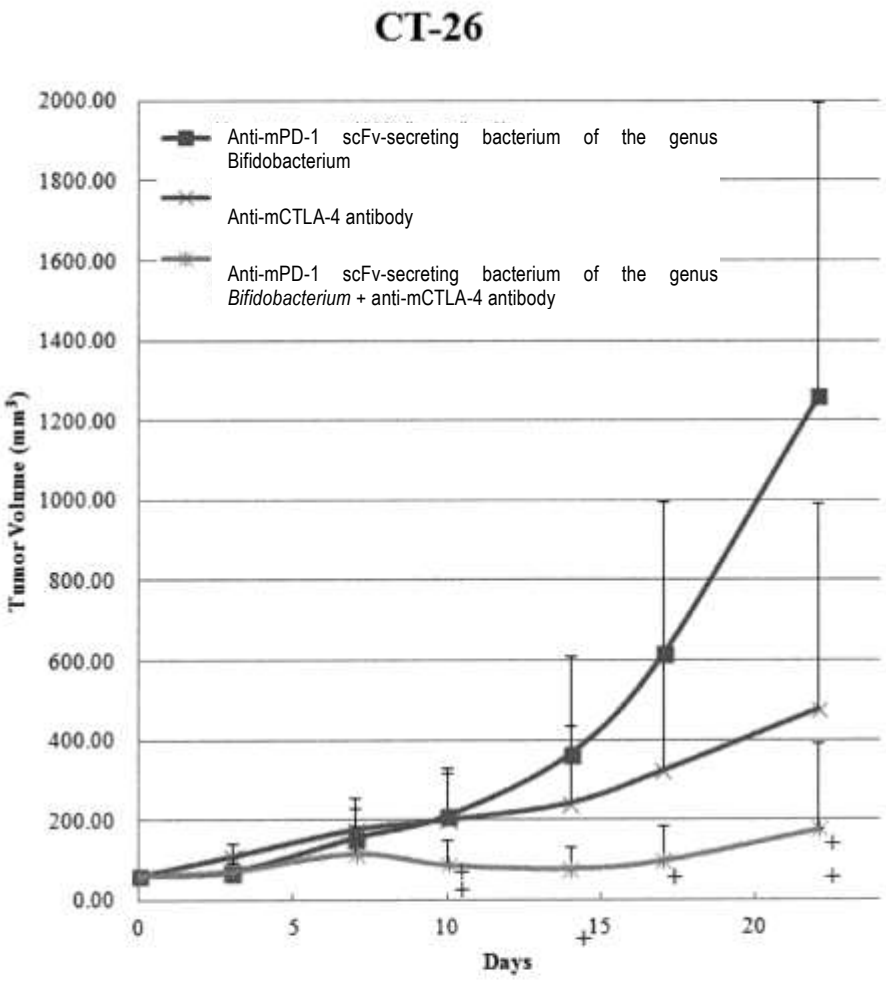


The mean values + S.D. of seven or six mice.

*: P value < 0.05 (vs Non-treated)

**: P value < 0.01 (vs Non-treated)

[Figure 20]



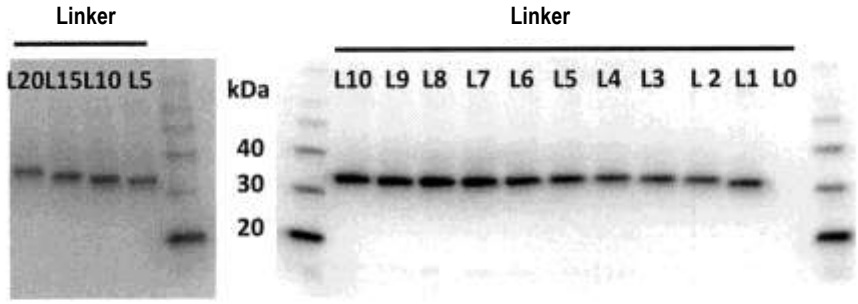
The mean values + S.D. of seven or six animals

+: P value < 0.05 (vs combination treatment)

++: P value < 0.01 (vs combination treatment)

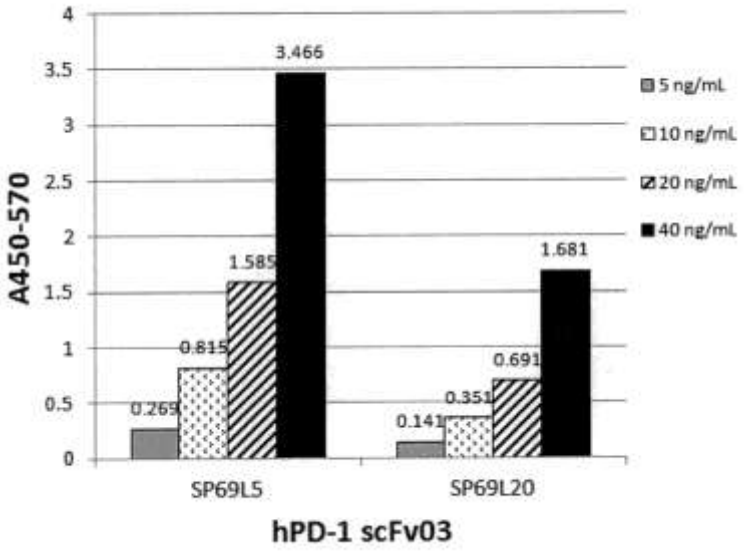
[Figure 21]

Bifidobacterium longum 105-A/pHuSP69Ly-hPD-1scFv03 strain

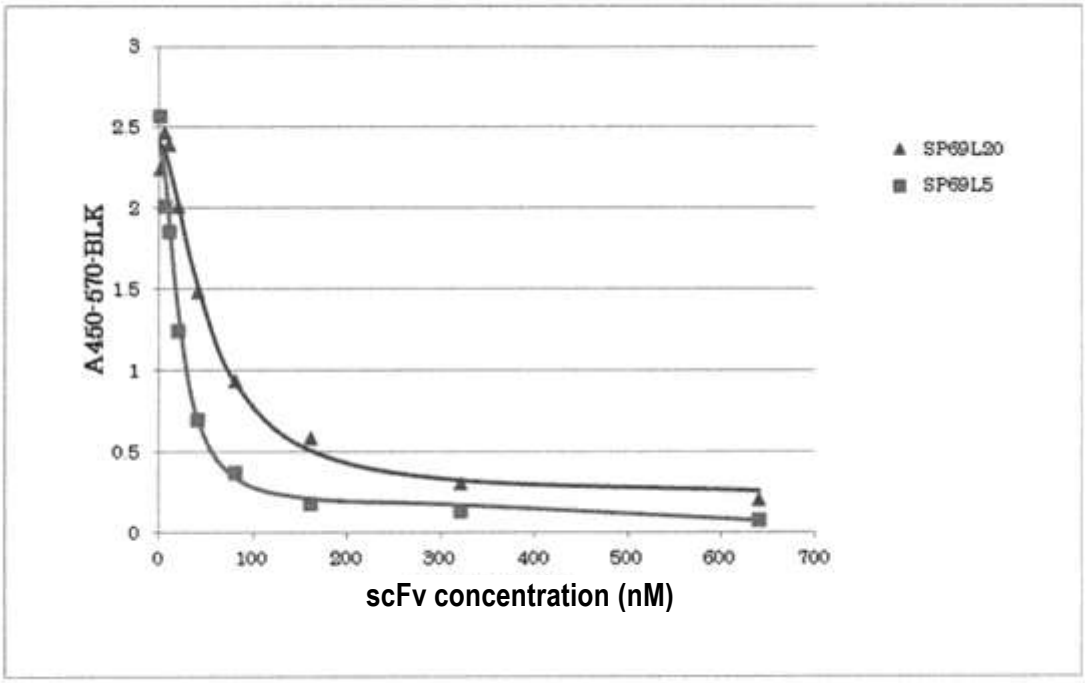


Western analysis of anti-hPD-1 scFv03 antibodies different in linker length secreted in supernatant of bacterium of the genus *Bifidobacterium* culture -

[Figure 22]



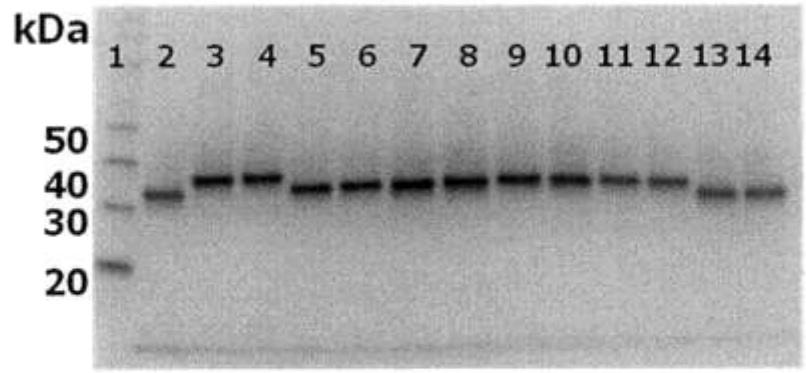
[Figure 23]


A450-570-BLK
scFv concentration (nM)
SP69L20
SP69L5
0
0.5
1
1.5
2
2.5
3
0
100
200
300
400
500
600
700

[Figure 24]

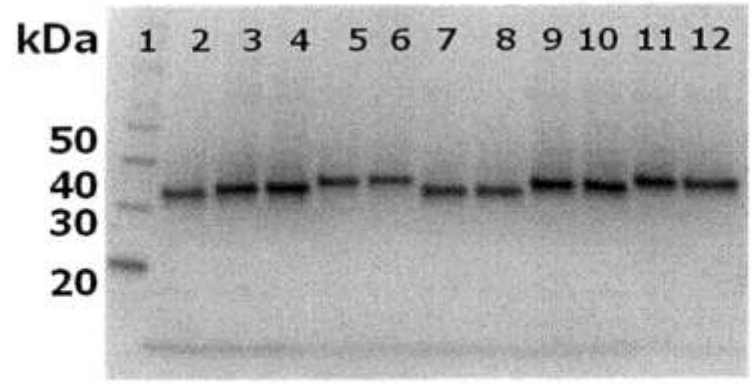
(a)

B. longum 105A/pHuSPxL20-hPD-1 scFv03



1: Marker
2: SP7
3: SP45 #1
4: SP45 #2
5:
6:
7: SP50 #1
8: SP50 #2
9: SP52 #1
10: SP52 #2
11: SP55 #1
12: SP55 #2
13: SP58 #1
14: SP58 #2

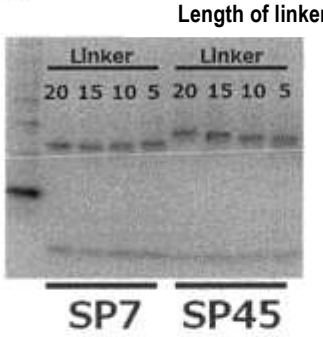
(b)



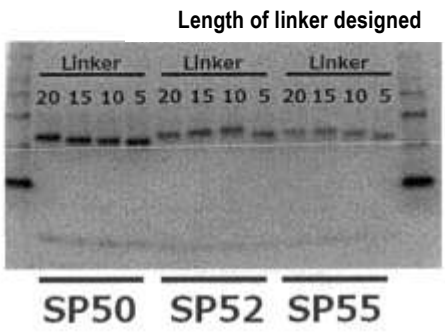
1: Marker
2: SP7
3: SP64 #1
4: SP64 #2
5: SP66 #1
6: SP66 #2
7: SP67 #1
8: SP67 #2
9: SP68 #1
10: SP68 #2
11: SP69 #1
12: SP69 #2

[Figure 25]

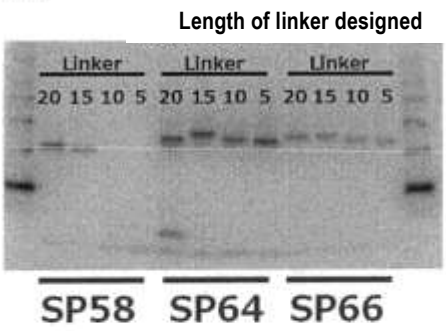
(a)



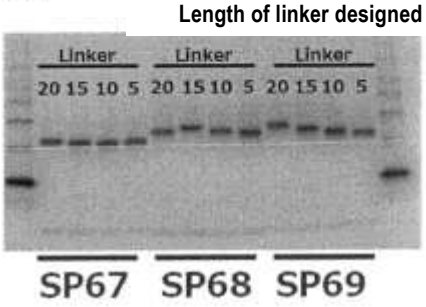
(b)



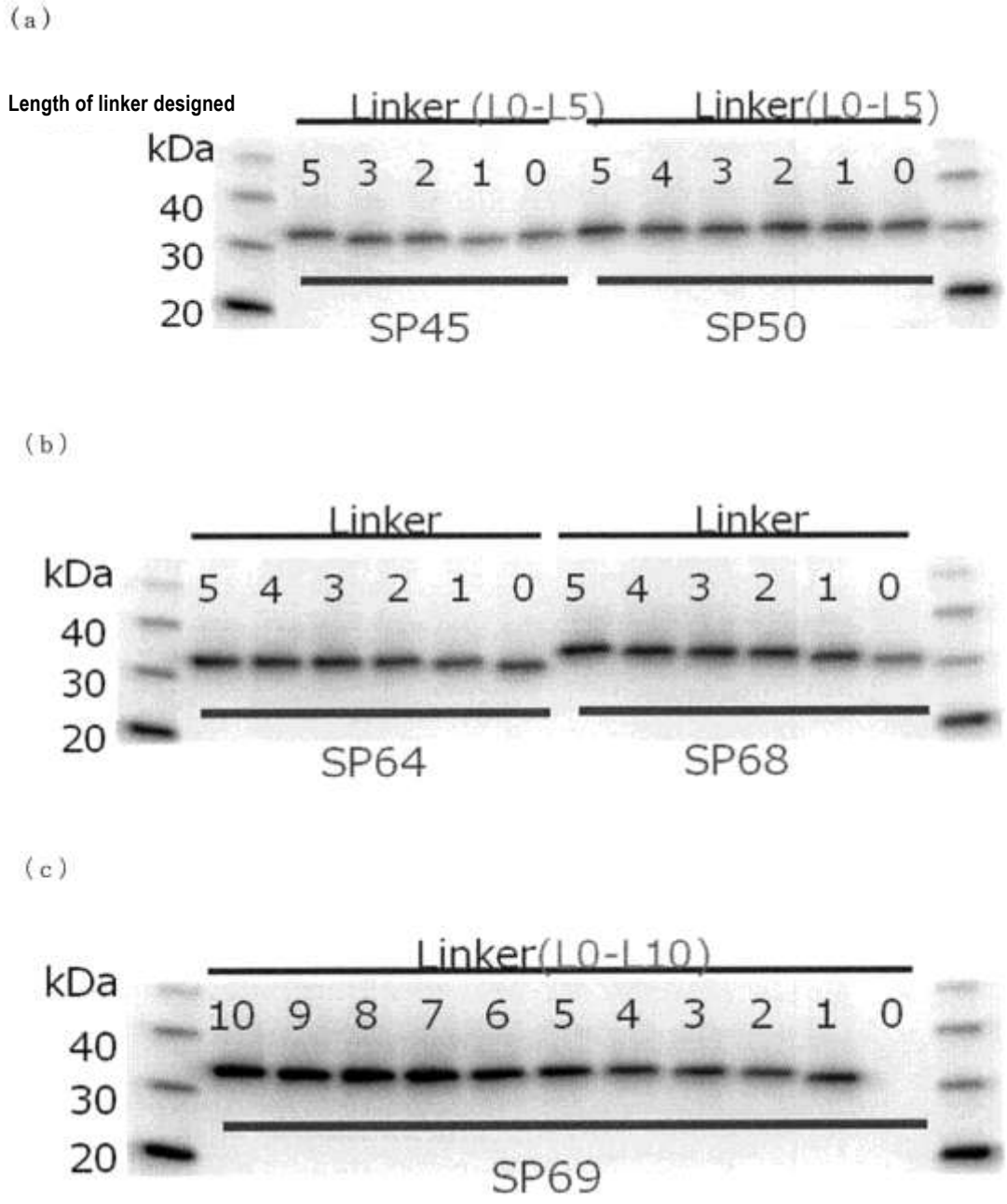
(c)



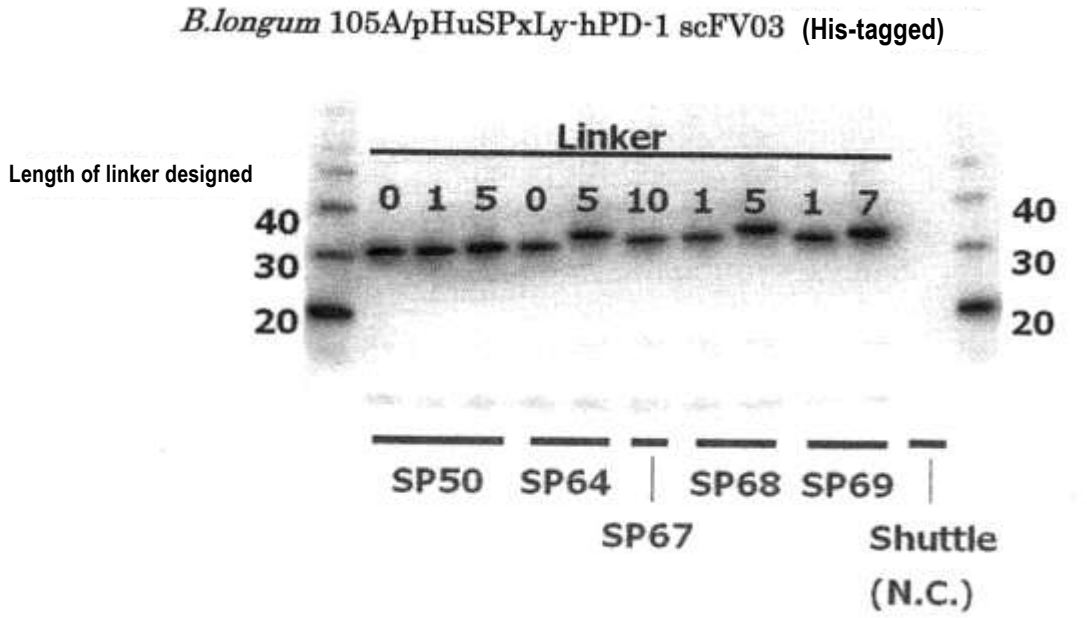
(d)



[Figure 26]

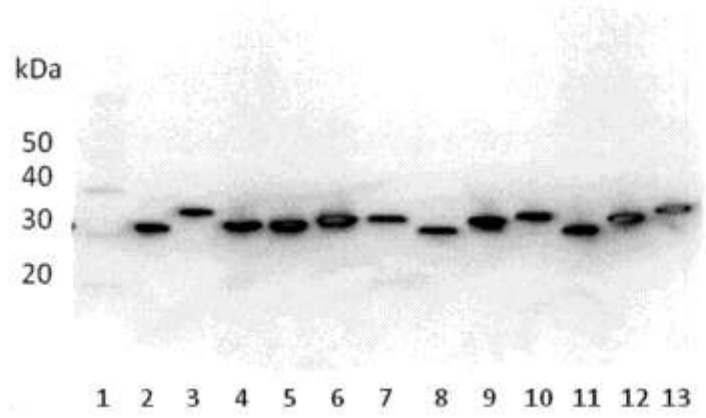

(a)
Length of linker designed
Linker (L0-L5)
Linker(L0-L5)
kDa
5 3 2 1 0 5 4 3 2 1 0
40
30
20
SP45
SP50
(b)
Linker
Linker
kDa
5 4 3 2 1 0 5 4 3 2 1 0
40
30
20
SP64
SP68
(c)
Linker(L0-L10)
kDa
10 9 8 7 6 5 4 3 2 1 0
40
30
20
SP69

[Figure 27]



[Figure 28]

Bifidobacterium longum 105-A/pP30SPxL20-hCTLA-4scFv02 strain



1: Marker
2: SP7
3: SP45
4:
5: SP50
6: SP52
7: SP55
8: SP58
9: SP64
10: SP66
11: SP67
12: SP68
13: SP69

[Figure 29]

Bifidobacterium longum 105-A/pP30SPxLy-hCTLA-4scFv02 strain (FLAG-tagged)

