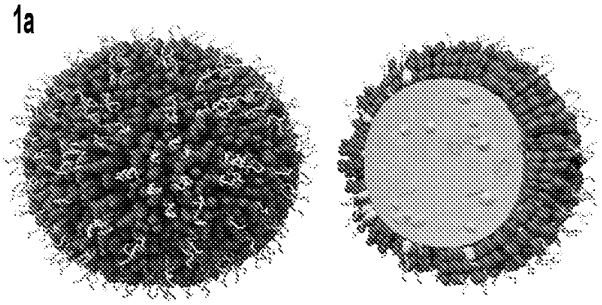
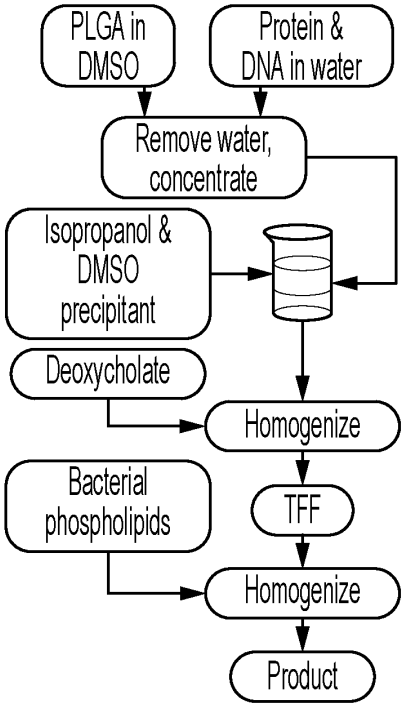
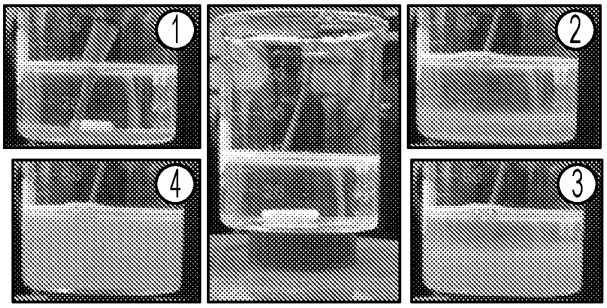
1a



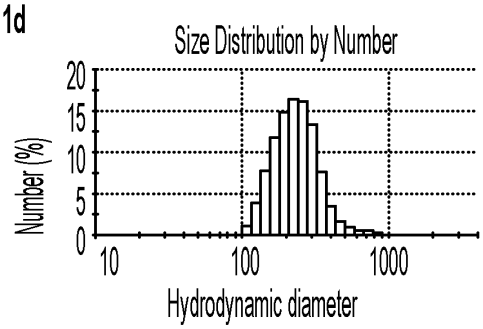
1b



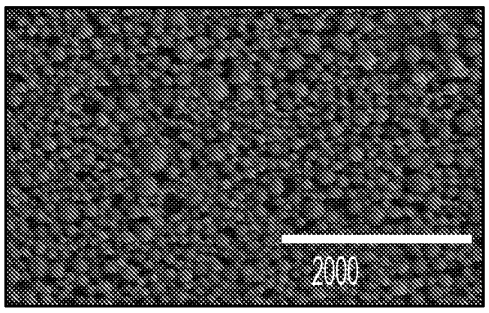
1c



1d



1e



1f

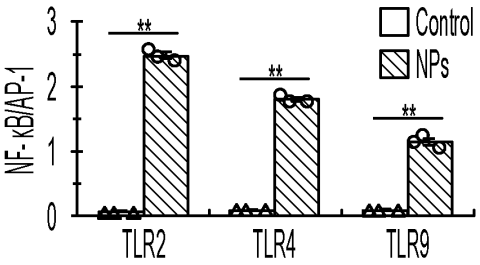


Figure 1

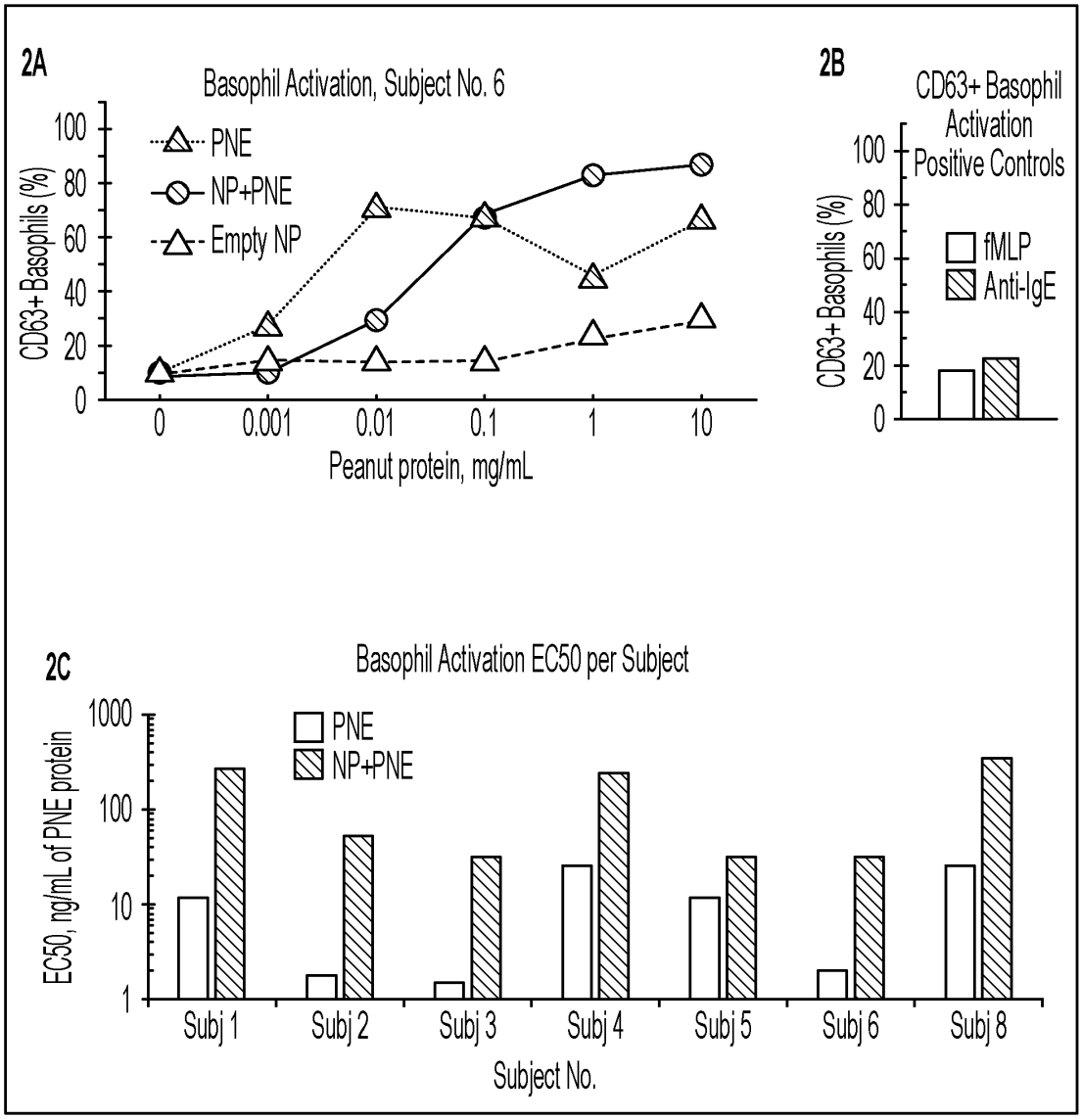

2A
Basophil Activation, Subject No. 6
CD63+ Basophils (%)
100
80
60
40
20
0
PNE
NP+PNE
Empty NP
0
0.001
0.01
0.1
1
10
Peanut protein, mg/mL
2B
CD63+ Basophil Activation Positive Controls
CD63+ Basophils (%)
100
80
60
40
20
0
fMLP
Anti-IgE
2C
Basophil Activation EC50 per Subject
EC50, ng/mL of PNE protein
1000
100
10
1
PNE
NP+PNE
Subj 1
Subj 2
Subj 3
Subj 4
Subj 5
Subj 6
Subj 8
Subject No.

Figure 2

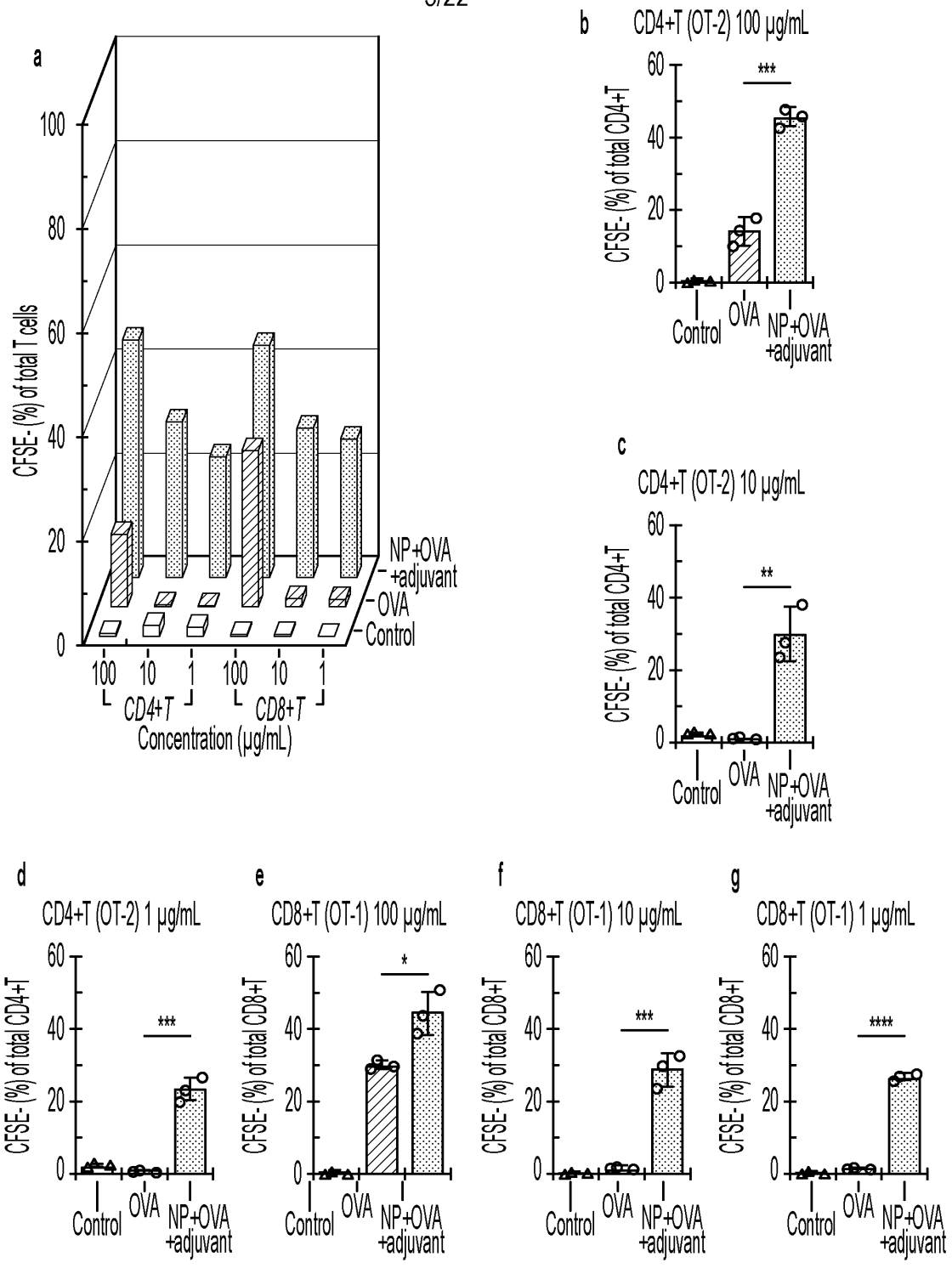

a
CFSE- (%) of total T cells
100
80
60
40
20
0
NP+OVA +adjuvant
OVA
Control
100 10 1 100 10 1
CD4+T
CD8+T
Concentration (μg/mL)
b
CD4+T (OT-2) 100 μg/mL
CFSE- (%) of total CD4+T
***
Control
OVA
NP+OVA +adjuvant
c
CD4+T (OT-2) 10 μg/mL
CFSE- (%) of total CD4+T
**
Control
OVA
NP+OVA +adjuvant
d
CD4+T (OT-2) 1 μg/mL
CFSE- (%) of total CD4+T
***
Control
OVA
NP+OVA +adjuvant
e
CD8+T (OT-1) 100 μg/mL
CFSE- (%) of total CD8+T
*
Control
OVA
NP+OVA +adjuvant
f
CD8+T (OT-1) 10 μg/mL
CFSE- (%) of total CD8+T
***
Control
OVA
NP+OVA +adjuvant
g
CD8+T (OT-1) 1 μg/mL
CFSE- (%) of total CD8+T
****
Control
OVA
NP+OVA +adjuvant

Figure 3

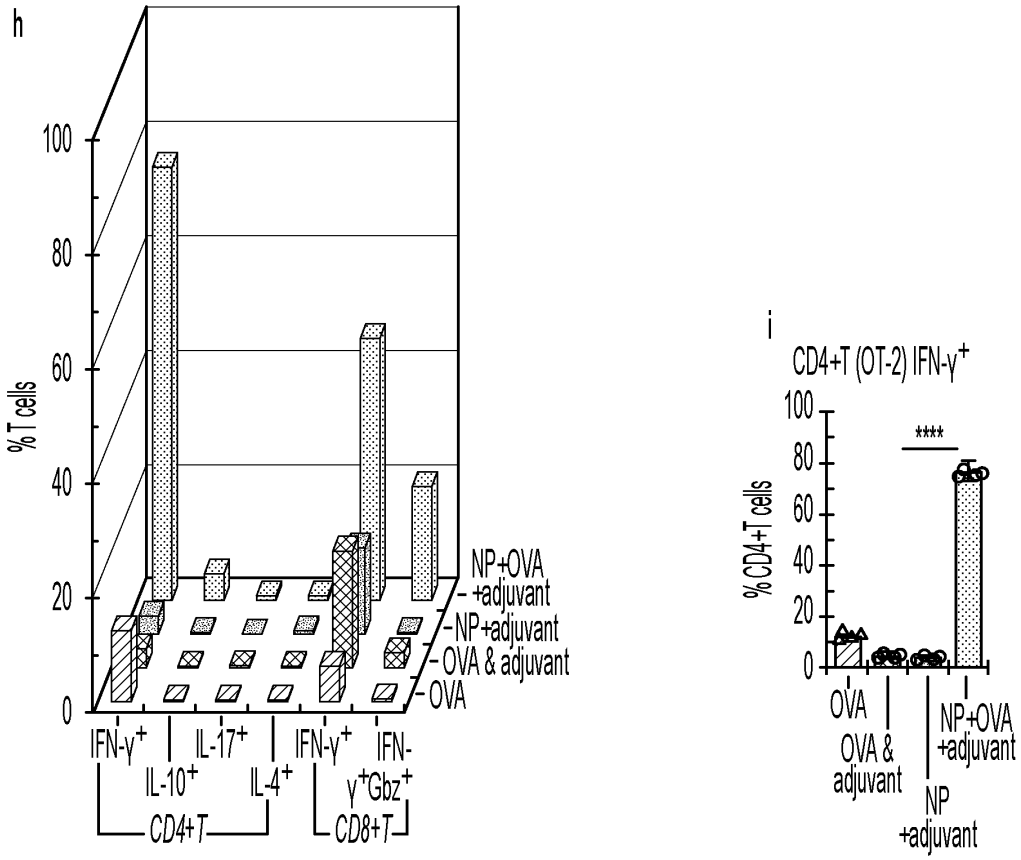


Figure 3
CONTINUED

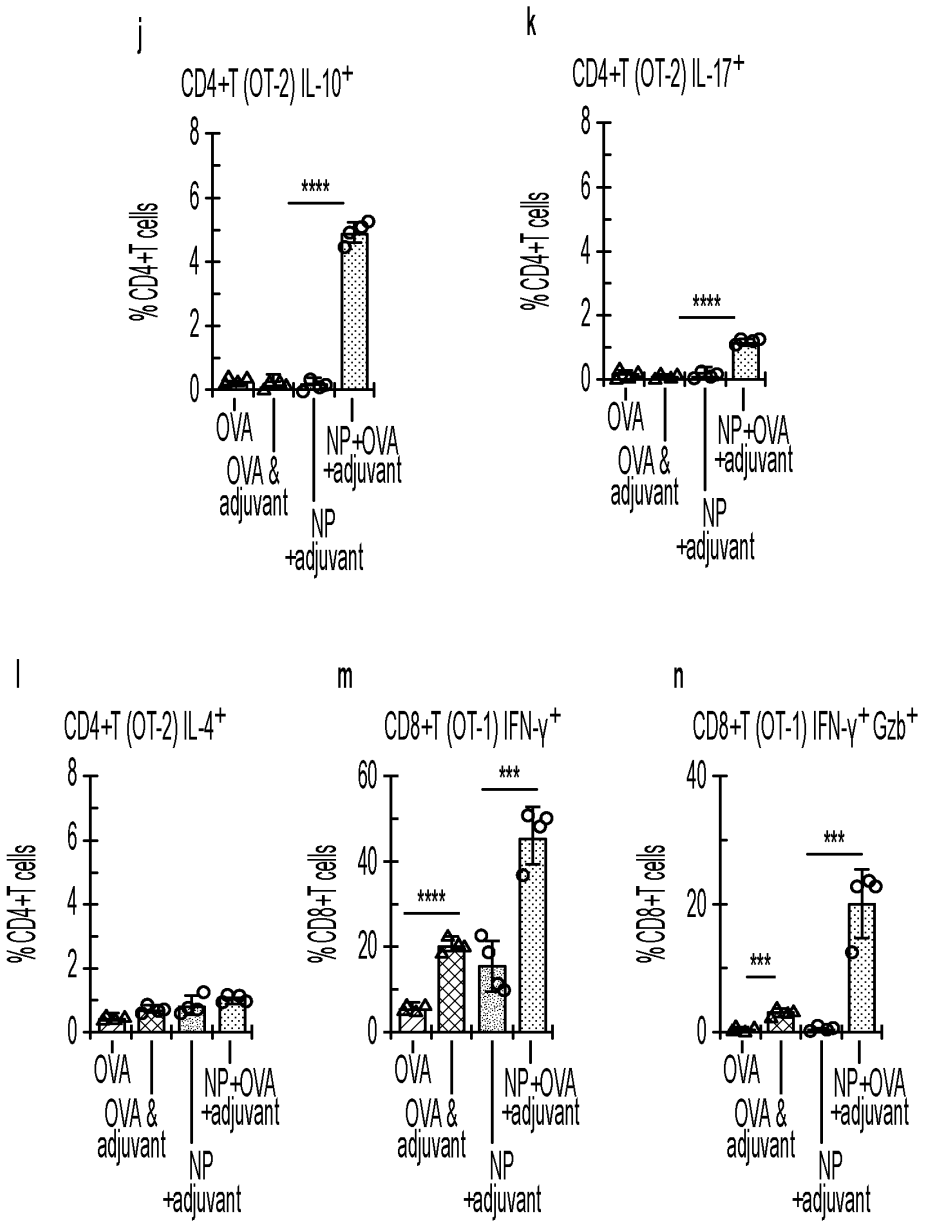


Figure 3
CONTINUED

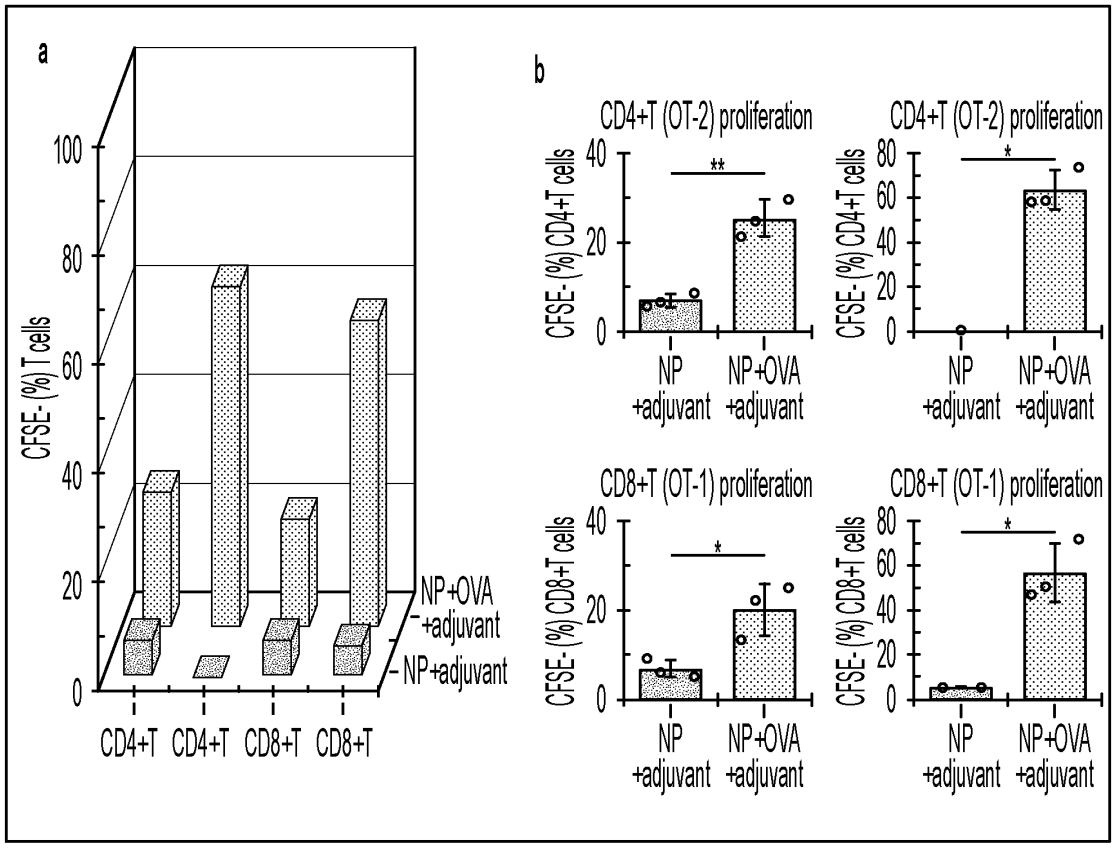

a
CFSE- (%) T cells
100
80
60
40
20
0
CD4+T
CD4+T
CD8+T
CD8+T
NP+OVA +adjuvant
NP+adjuvant
b
CD4+T (OT-2) proliferation
CFSE- (%) CD4+T cells
40
20
0
**
NP +adjuvant
NP+OVA +adjuvant
CD4+T (OT-2) proliferation
CFSE- (%) CD4+T cells
80
60
40
20
0
*
NP +adjuvant
NP+OVA +adjuvant
CD8+T (OT-1) proliferation
CFSE- (%) CD8+T cells
40
20
0
*
NP +adjuvant
NP+OVA +adjuvant
CD8+T (OT-1) proliferation
CFSE- (%) CD8+T cells
80
60
40
20
0
*
NP +adjuvant
NP+OVA +adjuvant

Figure 4

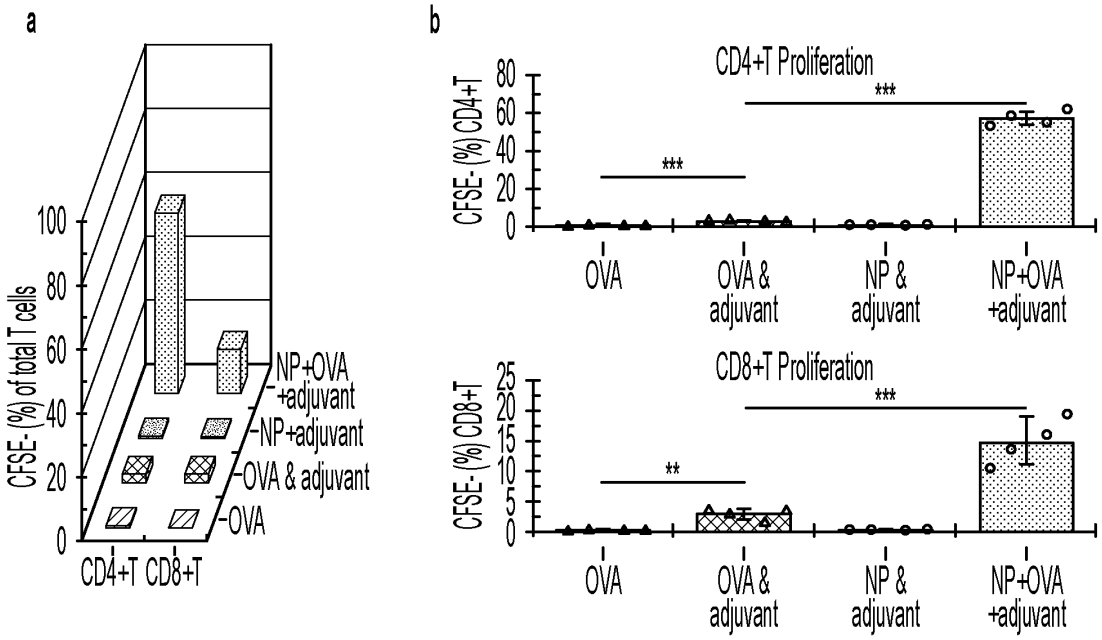

a
CFSE- (%) of total T cells
100
80
60
40
20
0
CD4+T CD8+T
NP+OVA +adjuvant
NP+adjuvant
OVA & adjuvant
OVA
b
CD4+T Proliferation
CFSE- (%) CD4+T
80
60
40
20
0
***
***
OVA
OVA & adjuvant
NP & adjuvant
NP+OVA +adjuvant
CD8+T Proliferation
CFSE- (%) CD8+T
25
20
15
10
5
0
**
***
OVA
OVA & adjuvant
NP & adjuvant
NP+OVA +adjuvant

Figure 5

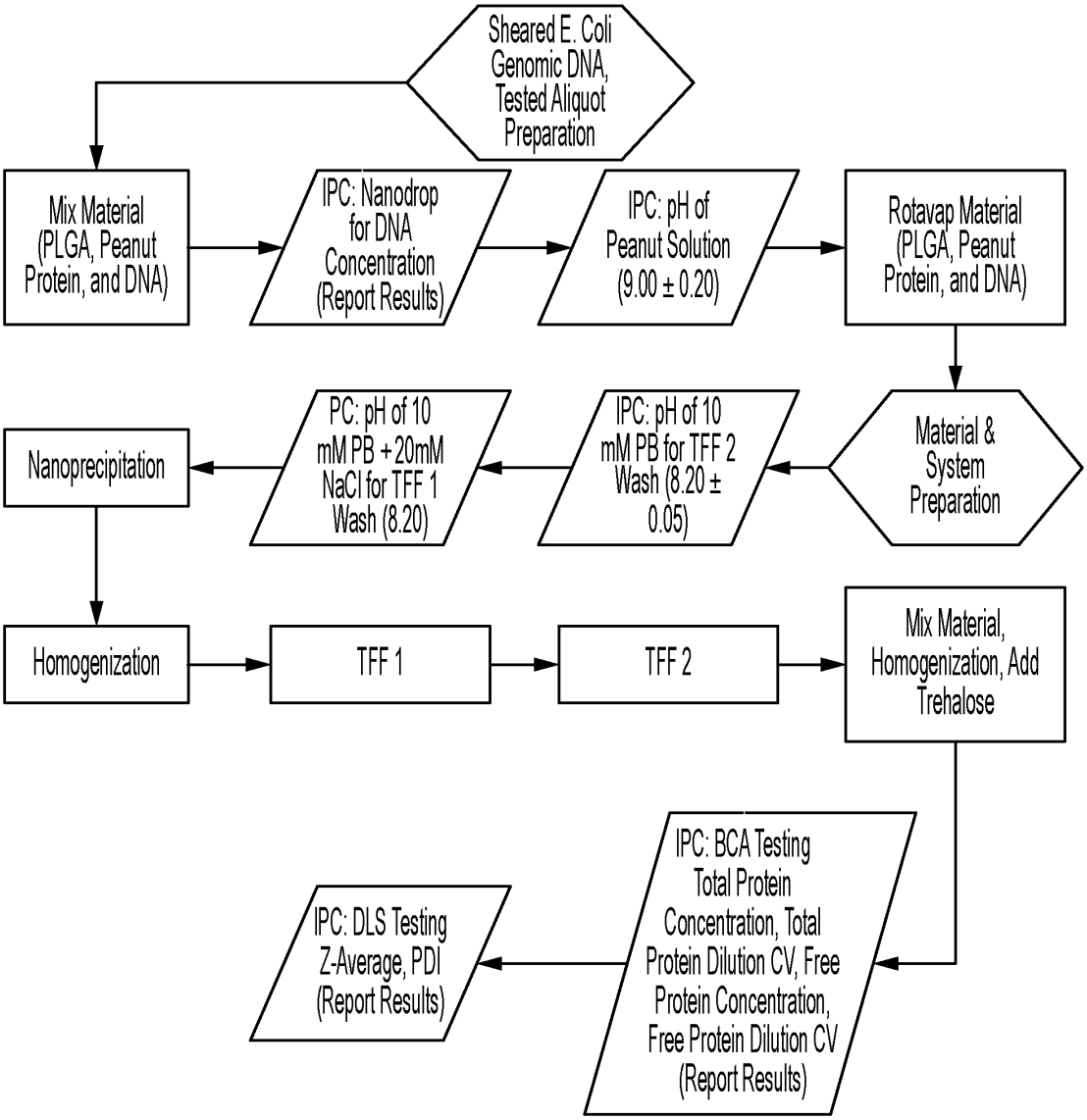

Sheared E. Coli Genomic DNA, Tested Aliquot Preparation
Mix Material (PLGA, Peanut Protein, and DNA)
IPC: Nanodrop for DNA Concentration (Report Results)
IPC: pH of Peanut Solution (9.00 ± 0.20)
Rotavap Material (PLGA, Peanut Protein, and DNA)
Material & System Preparation
IPC: pH of 10 mM PB for TFF 2 Wash (8.20 ± 0.05)
PC: pH of 10 mM PB +20mM NaCl for TFF 1 Wash (8.20)
Nanoprecipitation
Homogenization
TFF 1
TFF 2
Mix Material, Homogenization, Add Trehalose
IPC: BCA Testing Total Protein Concentration, Total Protein Dilution CV, Free Protein Concentration, Free Protein Dilution CV (Report Results)
IPC: DLS Testing Z-Average, PDI (Report Results)

Figure 6

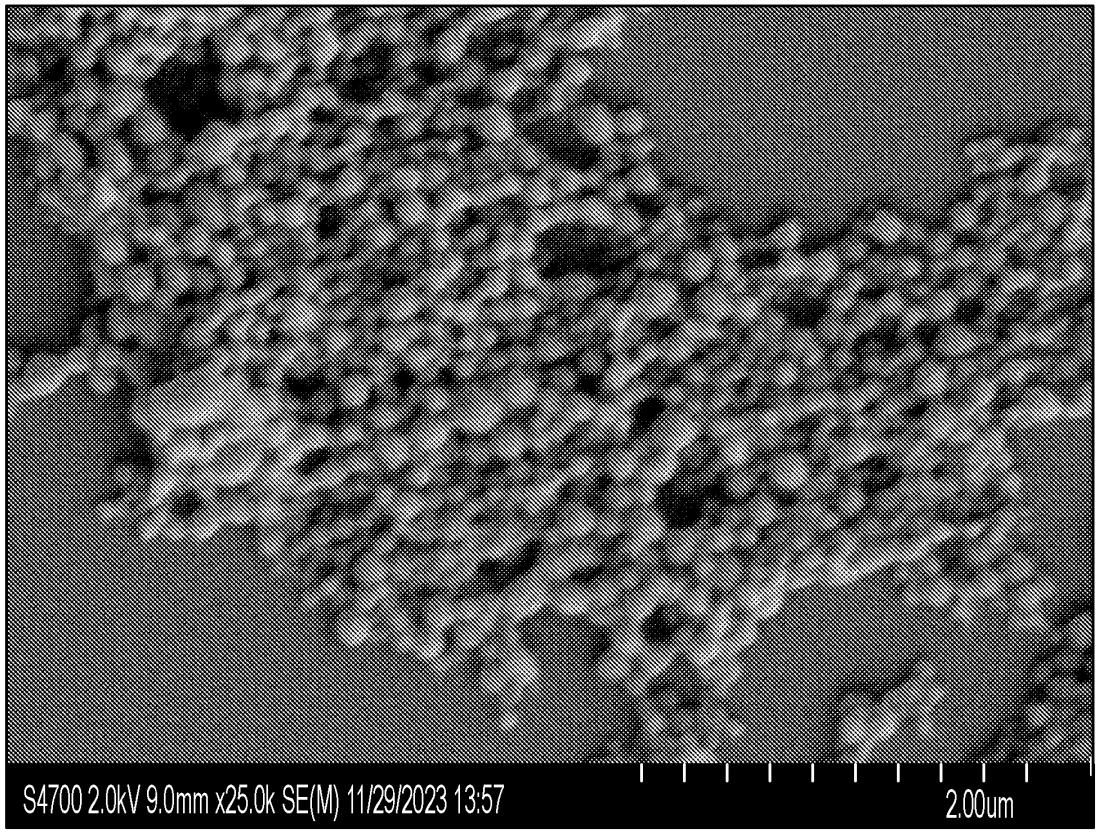


Figure 7

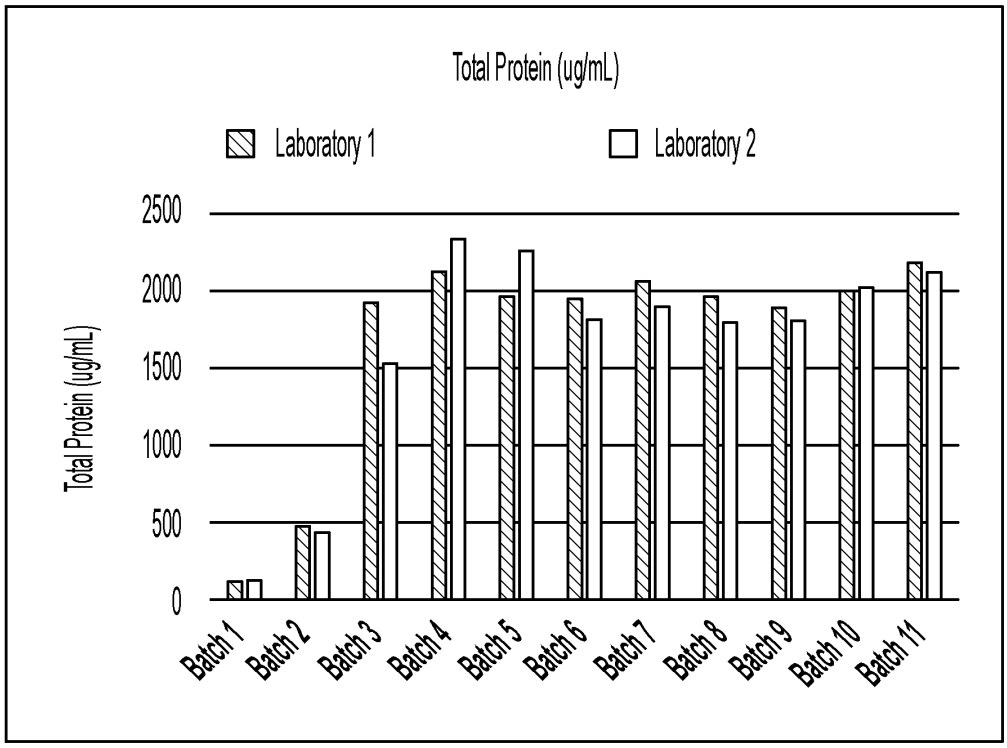

Total Protein (ug/mL)
Laboratory 1
Laboratory 2
Total Protein (ug/mL)
2500
2000
1500
1000
500
0
Batch 1
Batch 2
Batch 3
Batch 4
Batch 5
Batch 6
Batch 7
Batch 8
Batch 9
Batch 10
Batch 11

Figure 8

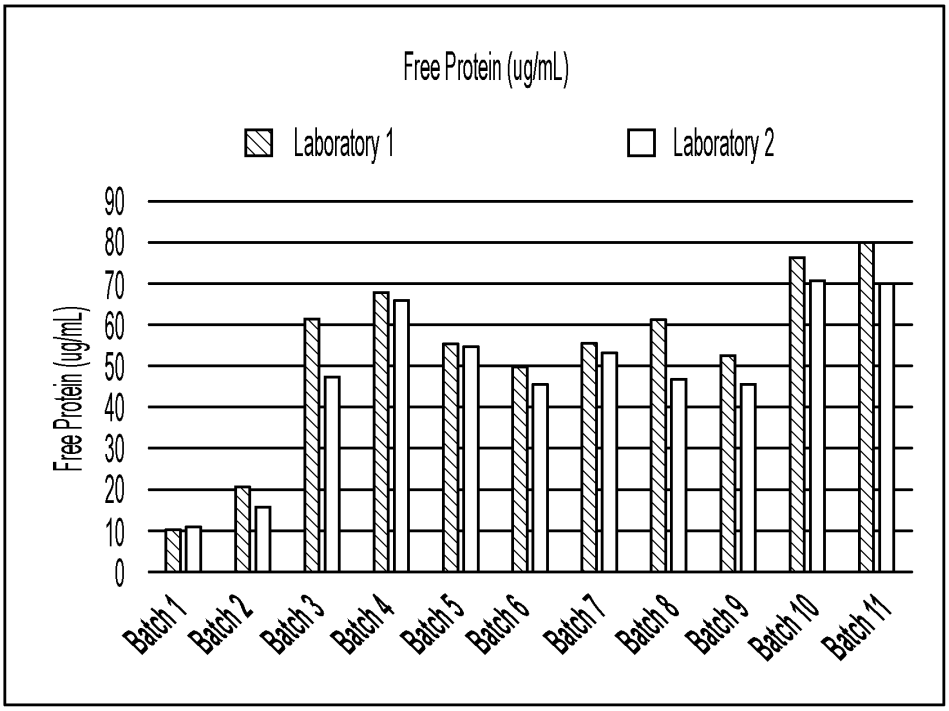

Free Protein (ug/mL)
Laboratory 1
Laboratory 2
Free Protein (ug/mL)
90
80
70
60
50
40
30
20
10
0
Batch 1
Batch 2
Batch 3
Batch 4
Batch 5
Batch 6
Batch 7
Batch 8
Batch 9
Batch 10
Batch 11

Figure 9

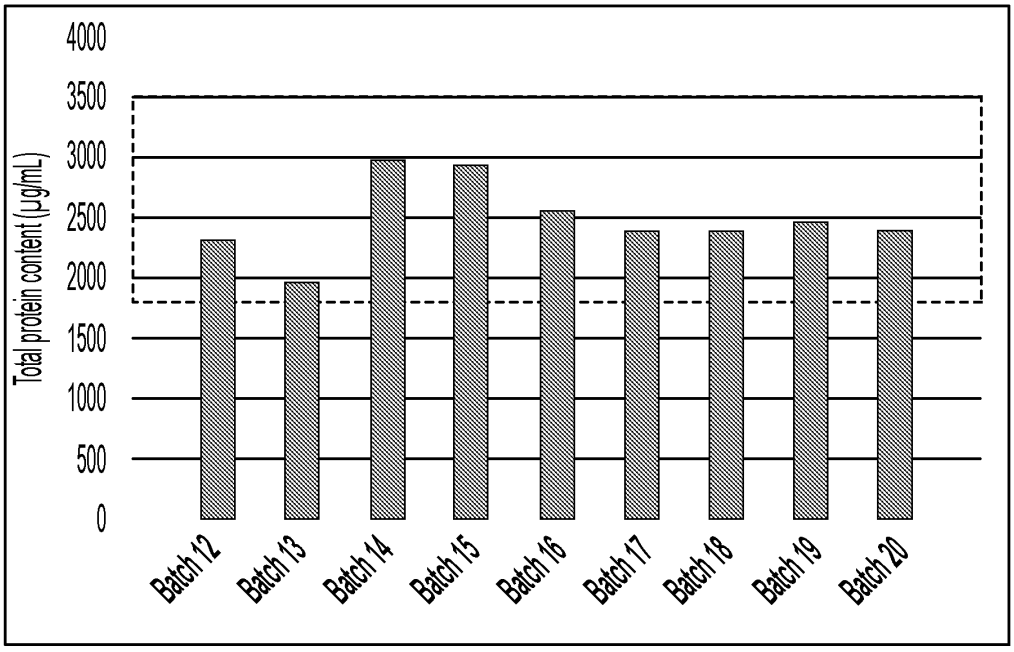

Total protein content (µg/mL)
4000
3500
3000
2500
2000
1500
1000
500
0
Batch 12
Batch 13
Batch 14
Batch 15
Batch 16
Batch 17
Batch 18
Batch 19
Batch 20

Figure 10

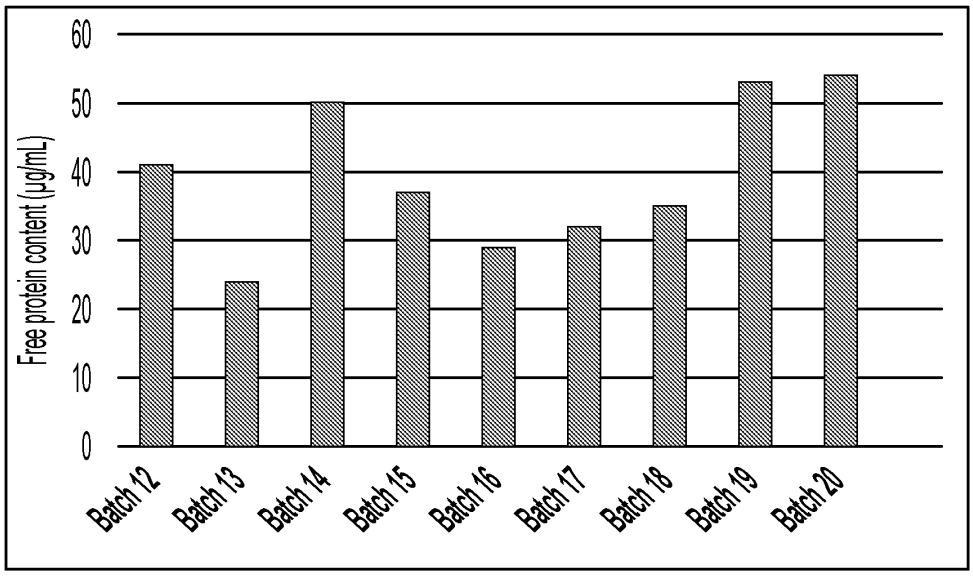

Free protein content (µg/mL)
60
50
40
30
20
10
0
Batch 12
Batch 13
Batch 14
Batch 15
Batch 16
Batch 17
Batch 18
Batch 19
Batch 20

Figure 11

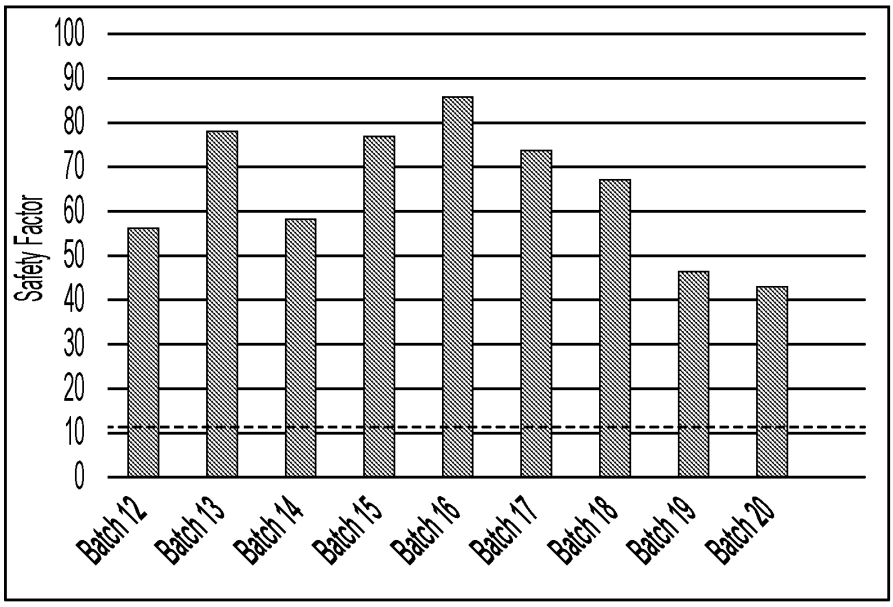

Safety Factor
100
90
80
70
60
50
40
30
20
10
0
Batch 12
Batch 13
Batch 14
Batch 15
Batch 16
Batch 17
Batch 18
Batch 19
Batch 20

Figure 12

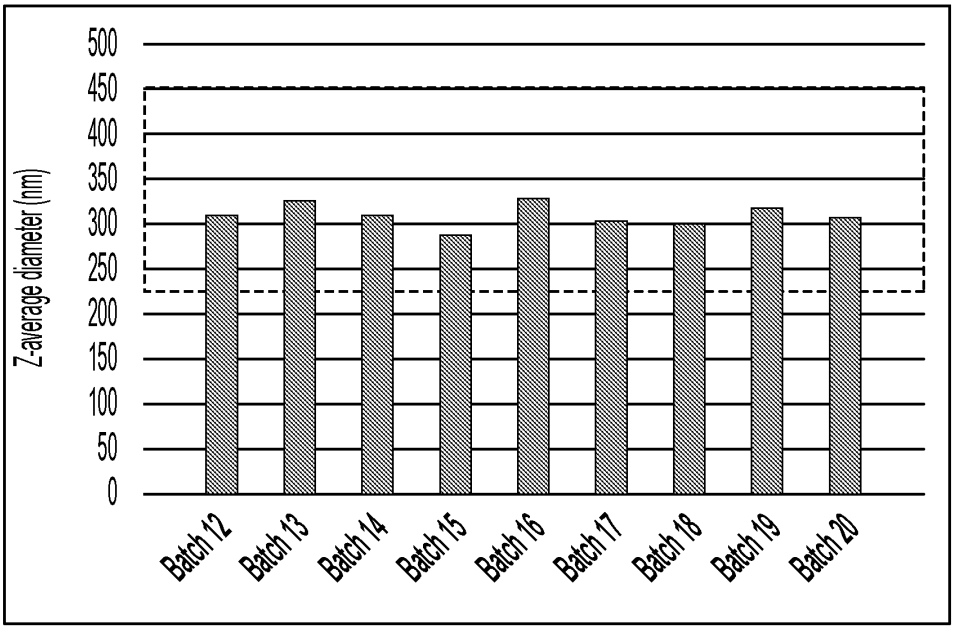

Z-average diameter (nm)
500
450
400
350
300
250
200
150
100
50
0
Batch 12
Batch 13
Batch 14
Batch 15
Batch 16
Batch 17
Batch 18
Batch 19
Batch 20

Figure 13

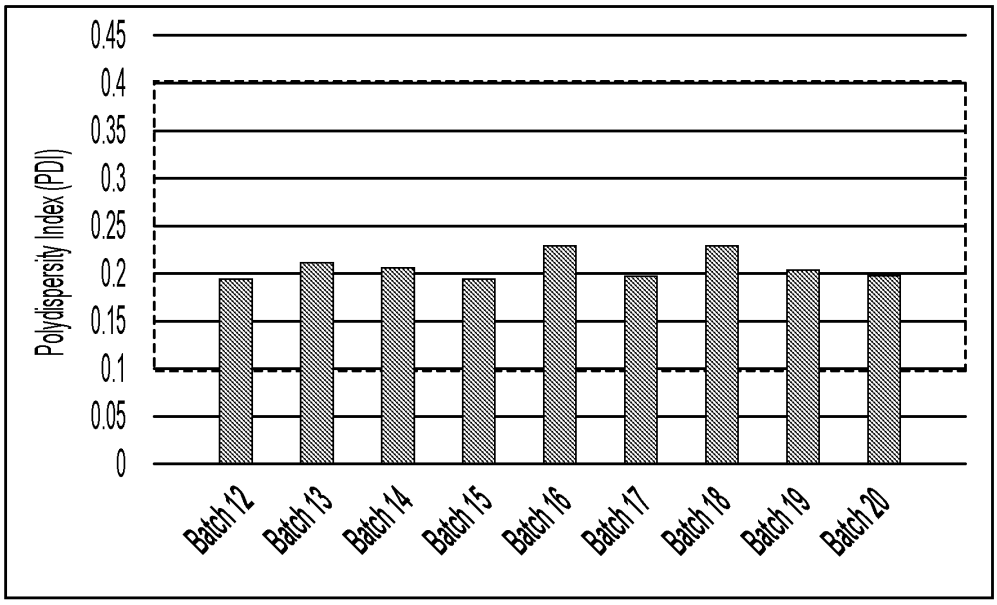

Polydispersity Index (PDI)
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Batch 12
Batch 13
Batch 14
Batch 15
Batch 16
Batch 17
Batch 18
Batch 19
Batch 20

Figure 14

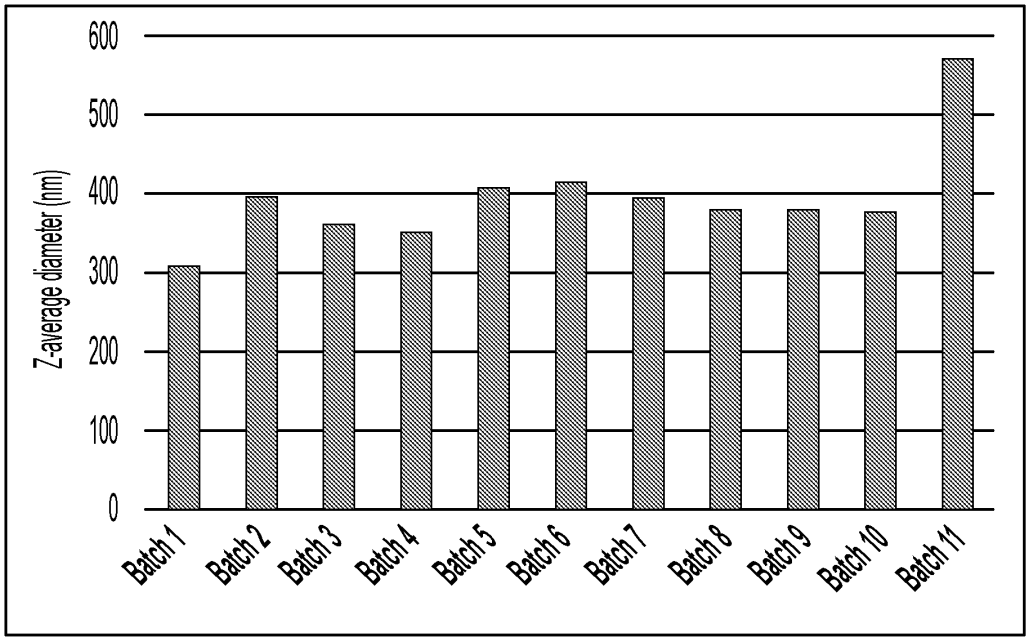

Z-average diameter (nm)
600
500
400
300
200
100
0
Batch 1
Batch 2
Batch 3
Batch 4
Batch 5
Batch 6
Batch 7
Batch 8
Batch 9
Batch 10
Batch 11

Figure 15

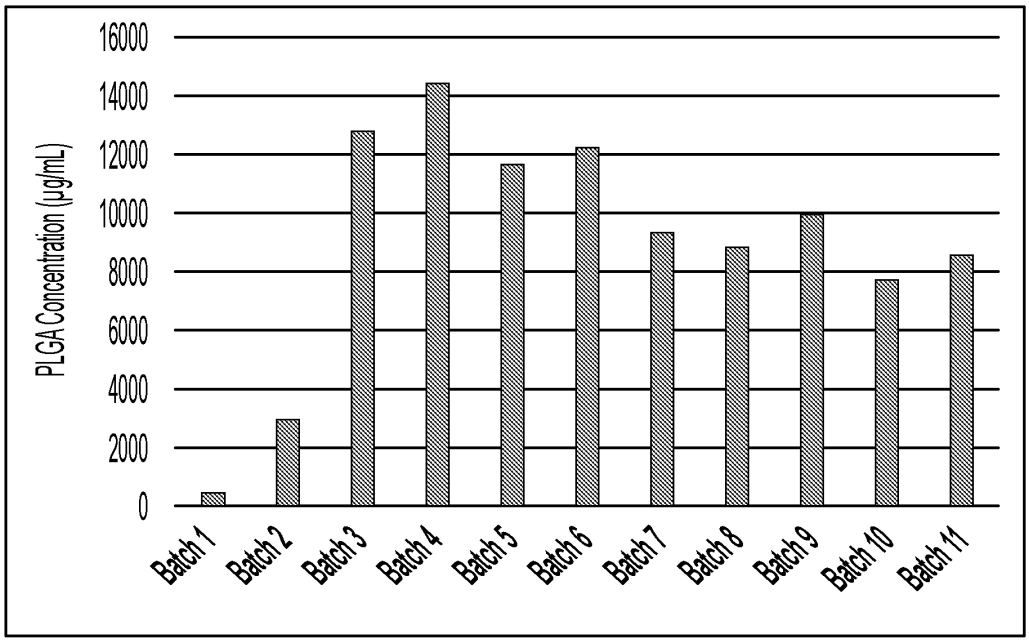

PLGA Concentration (µg/mL)
16000
14000
12000
10000
8000
6000
4000
2000
0
Batch 1
Batch 2
Batch 3
Batch 4
Batch 5
Batch 6
Batch 7
Batch 8
Batch 9
Batch 10
Batch 11

Figure 16

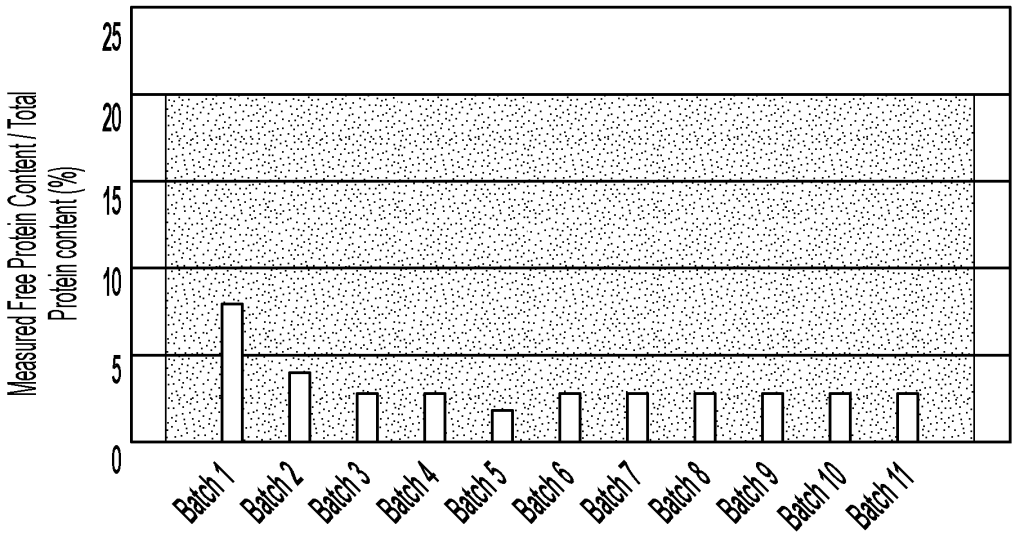

Measured Free Protein Content / Total Protein content (%)
25
20
15
10
5
0
Batch 1
Batch 2
Batch 3
Batch 4
Batch 5
Batch 6
Batch 7
Batch 8
Batch 9
Batch 10
Batch 11

Figure 17

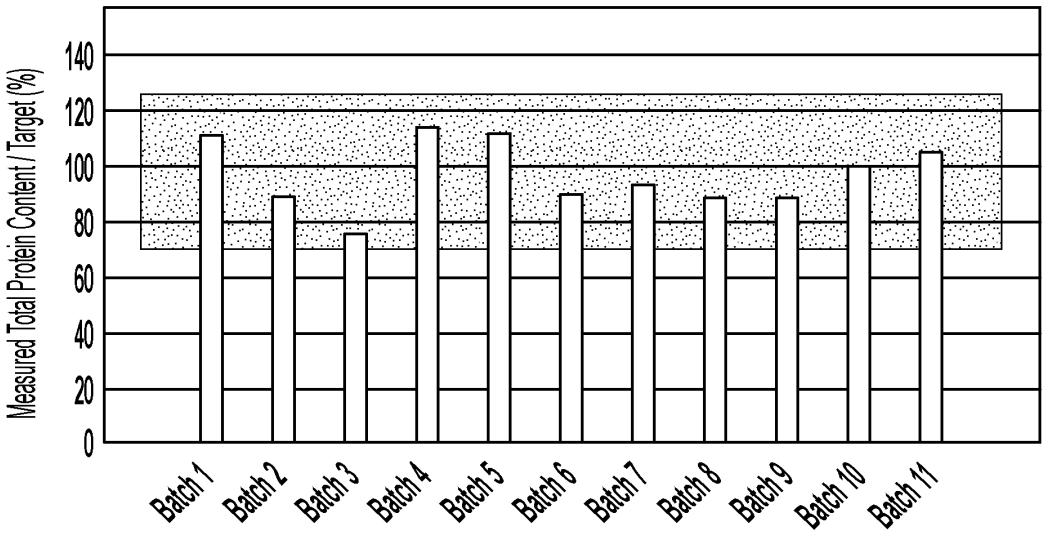

Measured Total Protein Content / Target (%)
140
120
100
80
60
40
20
0
Batch 1
Batch 2
Batch 3
Batch 4
Batch 5
Batch 6
Batch 7
Batch 8
Batch 9
Batch 10
Batch 11

Figure 18

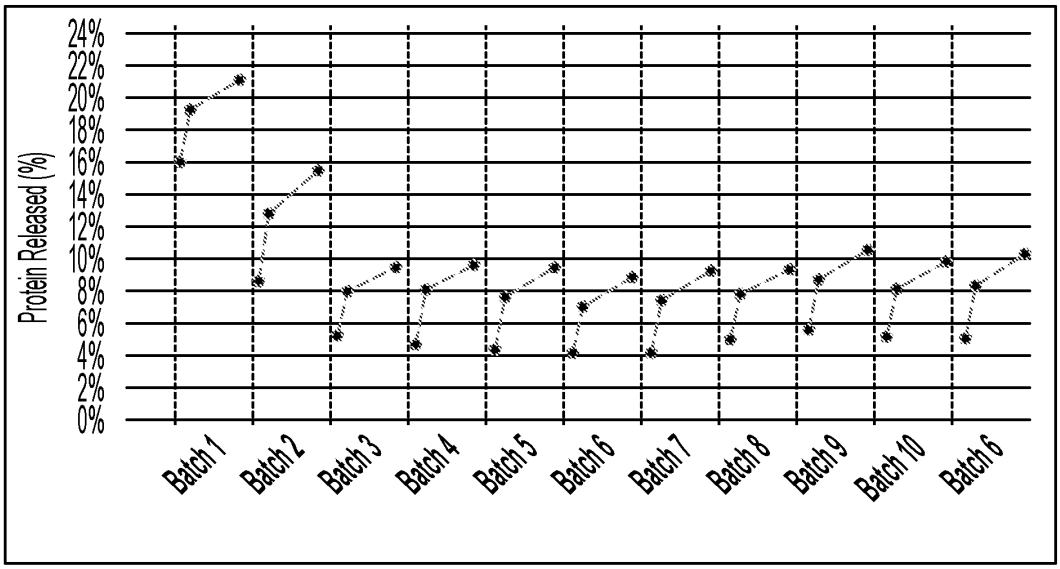

Protein Released (%)
24%
22%
20%
18%
16%
14%
12%
10%
8%
6%
4%
2%
0%
Batch 1
Batch 2
Batch 3
Batch 4
Batch 5
Batch 6
Batch 7
Batch 8
Batch 9
Batch 10
Batch 6

Figure 19

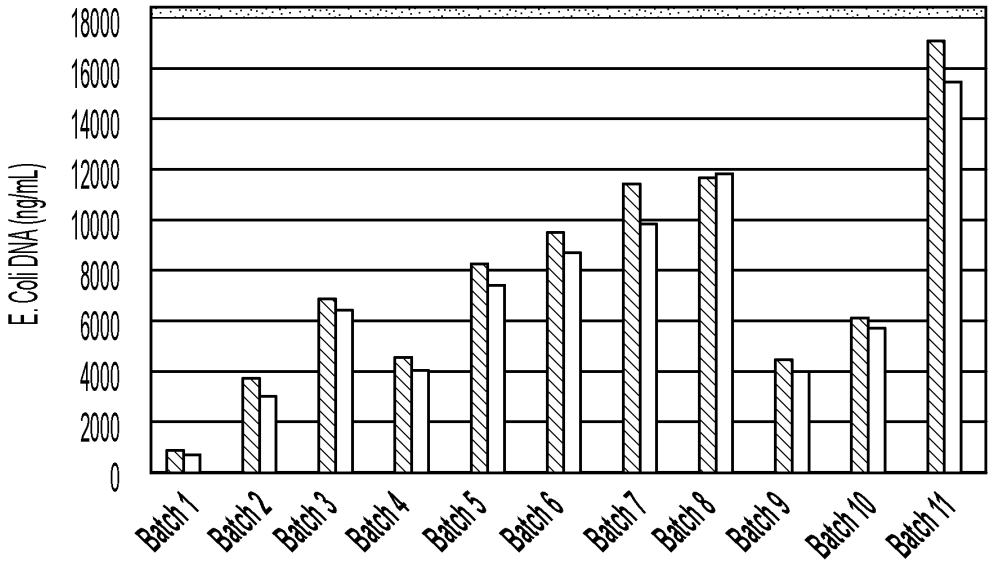

E. Coli DNA (ng/mL)
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
Batch 1
Batch 2
Batch 3
Batch 4
Batch 5
Batch 6
Batch 7
Batch 8
Batch 9
Batch 10
Batch 11

Figure 20