Super Plus
Serum Replacement Additive

FBS vs. NBCS+Super Plus
Challenge Test Result

Product Introduction

1. Super Plus serum replacement additive is designed to reduce or completely eliminate the supply of serum for cell culture. Most cell lines could be cultivated with their existed basal medium with reduced serum supply together with the Super Plus SFM. More than 80% to 90% serum could be reduced without affecting the cell growth performance. The product can reduce the culture variance due to the quality and consistency problems in serum and also reduce the cost from serum.
2. Most commercial-used cells have been tested with the comparison with standard serum-contained culture medium including VERO, MDCK, HEK239, BHK-21, MDBK,CHO, PK15, MAK, ST, and WI-38.
3. The following slides show the test result by three passages of cell culture in order to confirm the cell growth with the Super Plus serum replacement additive.

Materials

Basal Medium

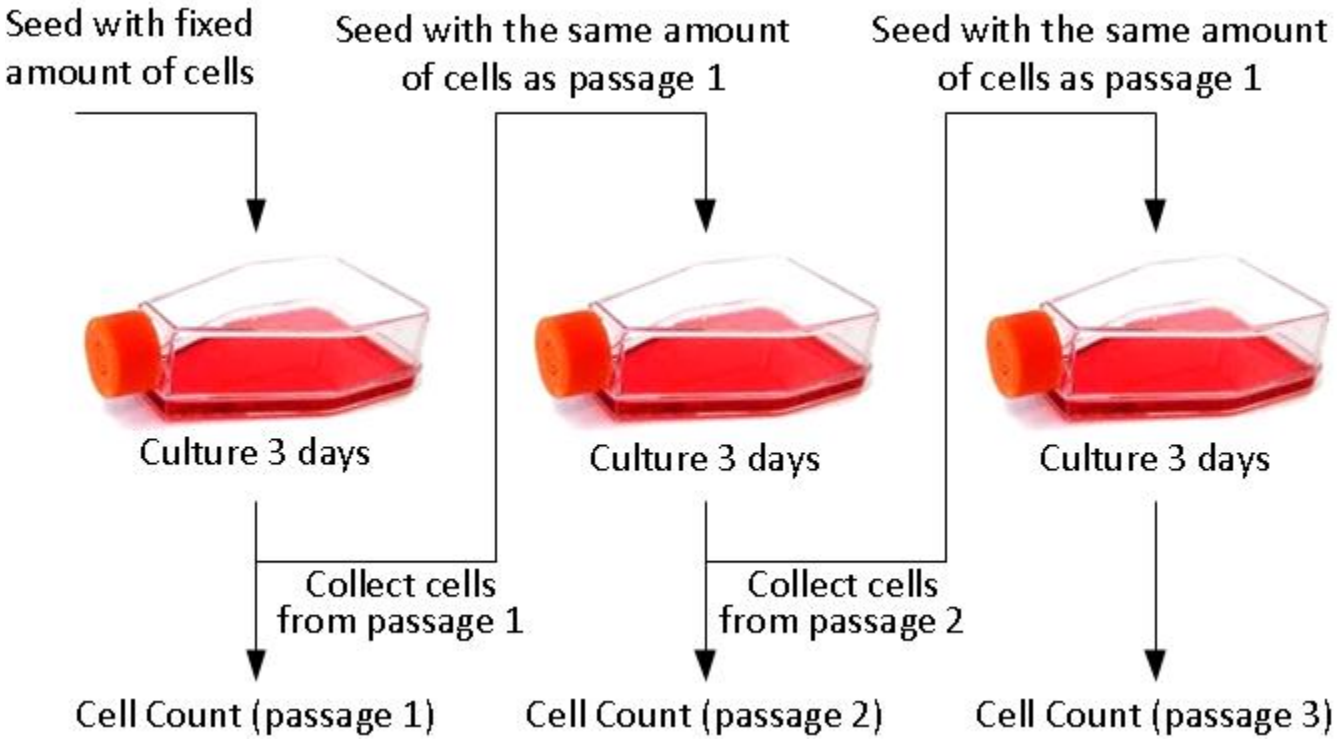
M199, F12, DMEM, E-MEM, alpha-MEM: Sigma Aldrich

Serum

1. Fetal Bovine Serum (FBS): ThermoFisher (Gibco), source from Australia
2. New Born Calf Serum (NBCS): LanZou, source from China (proven to be low quality serum as a negative control to compare with the test group that adding fetal bovine serum)
3. Super Plus (SP): CESCO Bioengineering, component-defined powder, animal component-free.

Test Protocol

Control and tested media were tested by inoculating with fixed amount of cells in 25 cm^2 tissue culture flasks and cultured for three days, and with continued subculture for three passages.



Cell Culture Test Experiment Design

Cell line	VERO			MDCK				MDBK			
Basal Medium	M199			α-MEM			M199	E-MEM	E-MEM	M199	
FBS	5%	0%	0%	10%	0%	0%	0%	10%	0%	0%	0%
New Born Calf Serum	0%	1%	0.5%	0%	1%	0.5%	1%	0%	1%	1%	0.5%
Super Plus	0 g	1.51 g	1.51 g	0 g	1.51 g	1.51 g	1.51 g	0 g	1.51 g	1.51 g	1.51 g

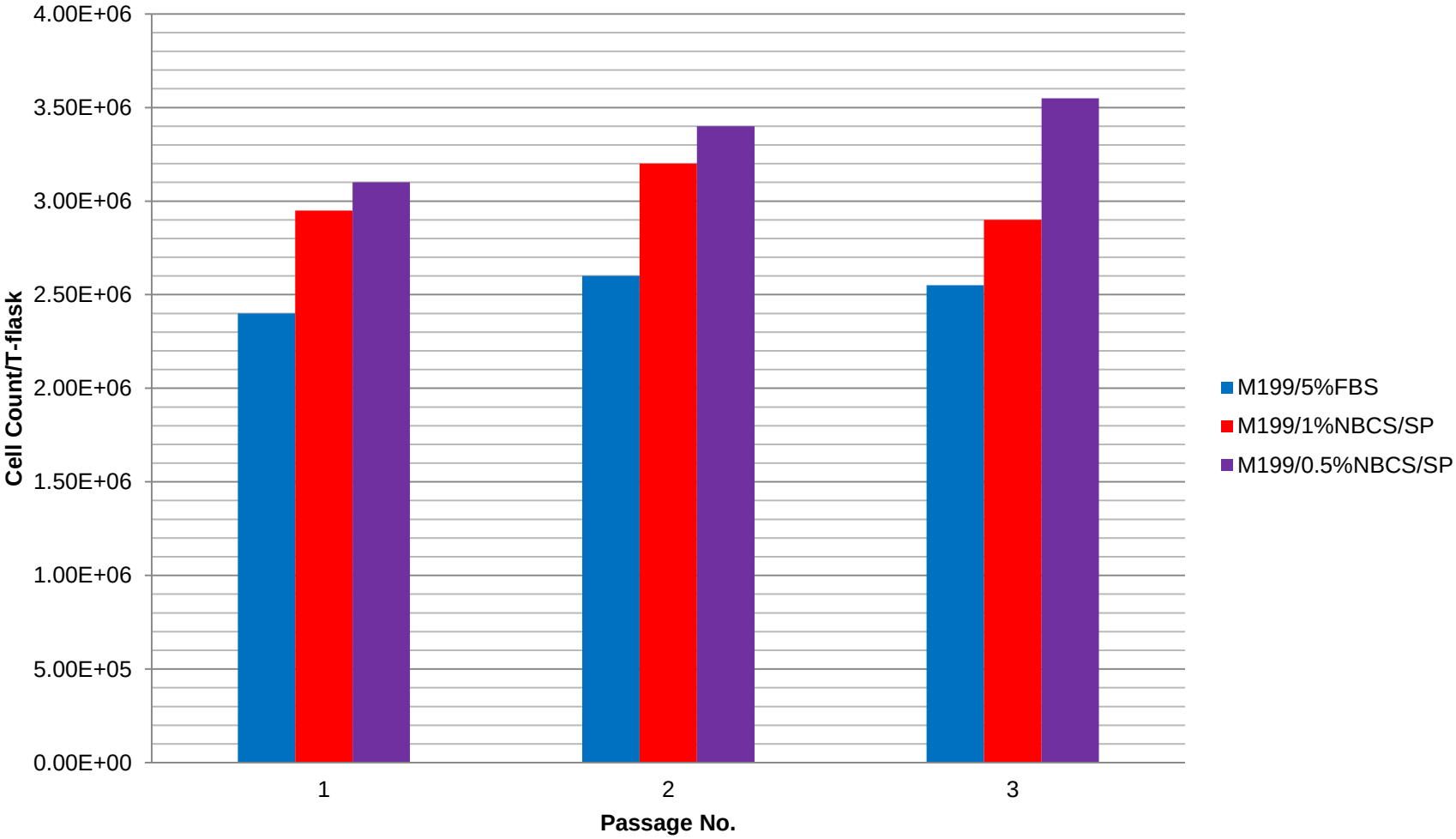
Cell line	CHO				HEK 293				BHK21			
Basal Medium	F-12		M199		a-MEM	a-MEM	M199		a-MEM	a-MEM	M199	
FBS	10%	0%	0%	0%	10%	0%	0%	0%	10%	0%	0%	0%
New Born Calf Serum	0%	1%	1%	0.5%	0%	1%	1%	0.5%	0%	1%	1%	0.5%
Super Plus	0 g	1.51 g	1.51 g	1.51 g	0 g	1.51 g	1.51 g	1.51 g	0 g	1.51 g	1.51 g	1.51 g

Cell Culture Test Experiment Design (cont.)

Cell line	MAK				ST				PK-15			
Basal Medium	a-MEM			M199	a-MEM		M199		E-MEM			M199
FBS	10%	0%	0%	0%	10%	0%	0%	0%	10%	0%	0%	0%
New Born Calf Serum	0%	1%	1%	1%	0%	1%	1%	1%	0%	1%	1%	1%
Super Plus	0 g	1.51 g	1.51 g	1.51 g	0 g	1.51 g	1.51 g	1.51 g	0 g	1.51 g	1.51 g	1.51 g

Test Result

VERO Cells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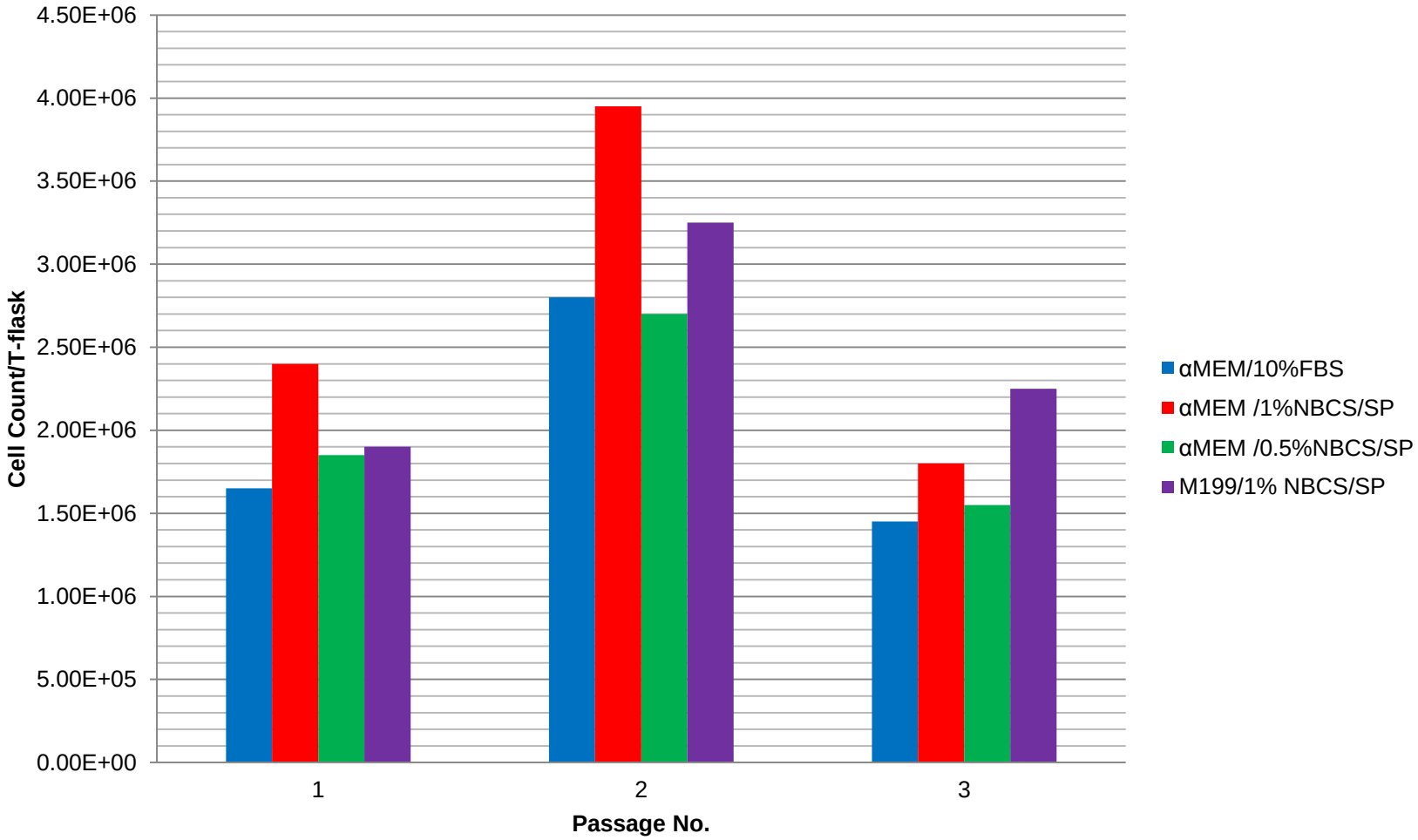
3.50E+06
3.00E+06
2.50E+06
2.00E+06
1.50E+06
1.00E+06
5.00E+05
0.00E+00
Cell Count/T-flask
1
2
3
Passage No.
M199/5%FBS
M199/1%NBCS/SP
M199/0.5%NBCS/SP

MDCK Cells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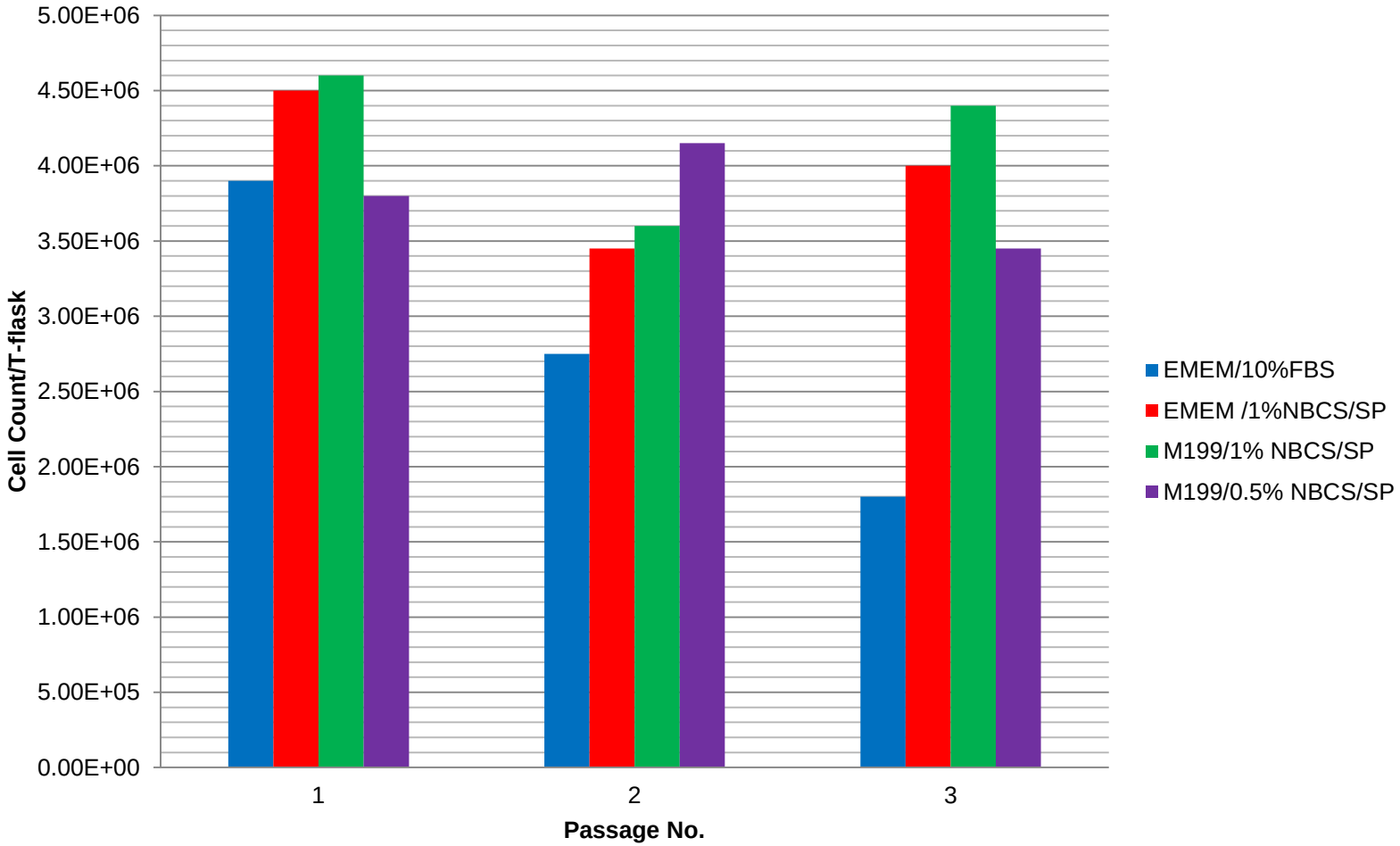
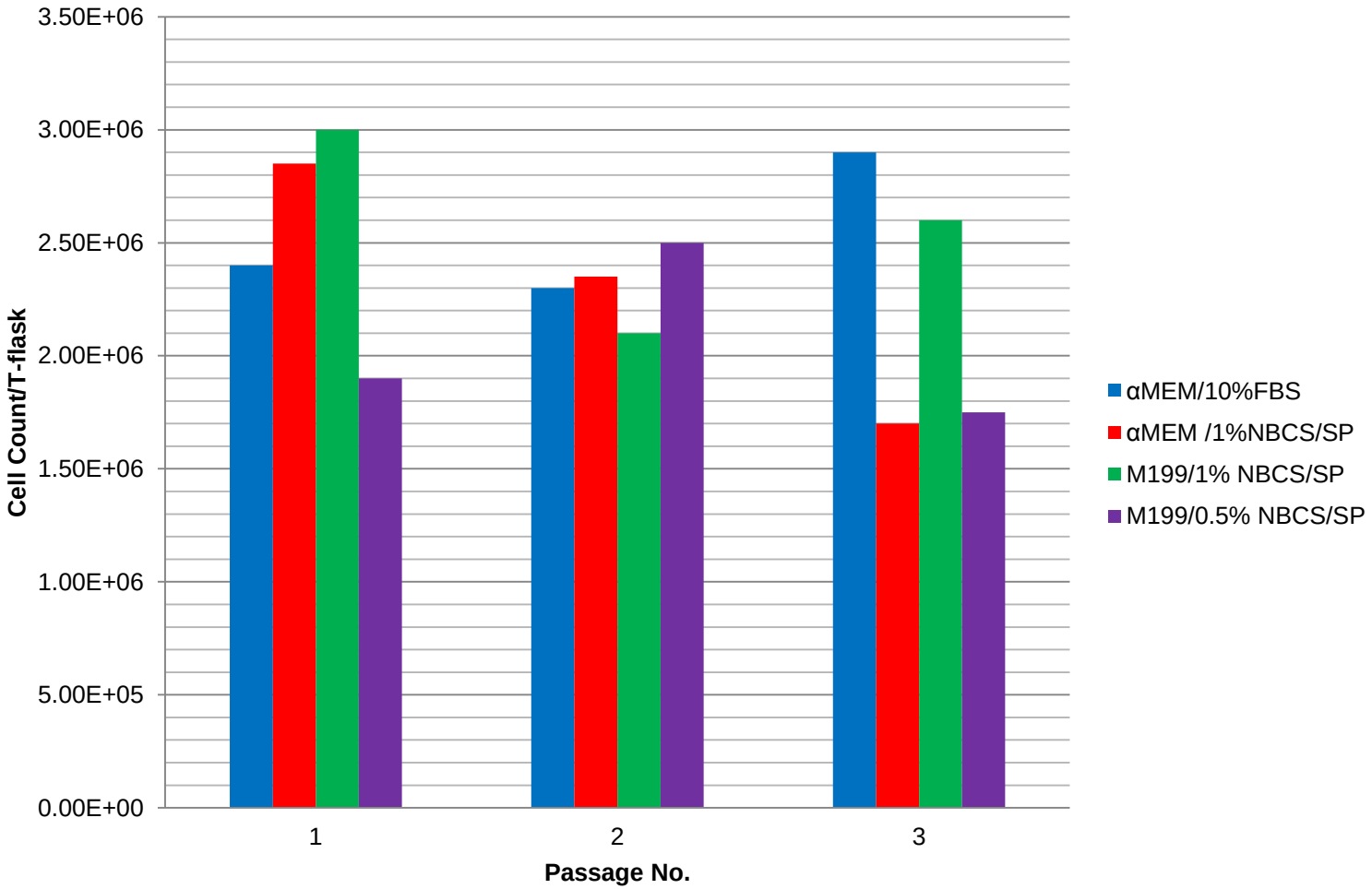
3.50E+06
3.00E+06
2.50E+06
2.00E+06
1.50E+06
1.00E+06
5.00E+05
0.00E+00
Cell Count/T-flask
1
2
3
Passage No.
αMEM/10%FBS
αMEM /1%NBCS/SP
αMEM /0.5%NBCS/SP
M199/1% NBCS/SP

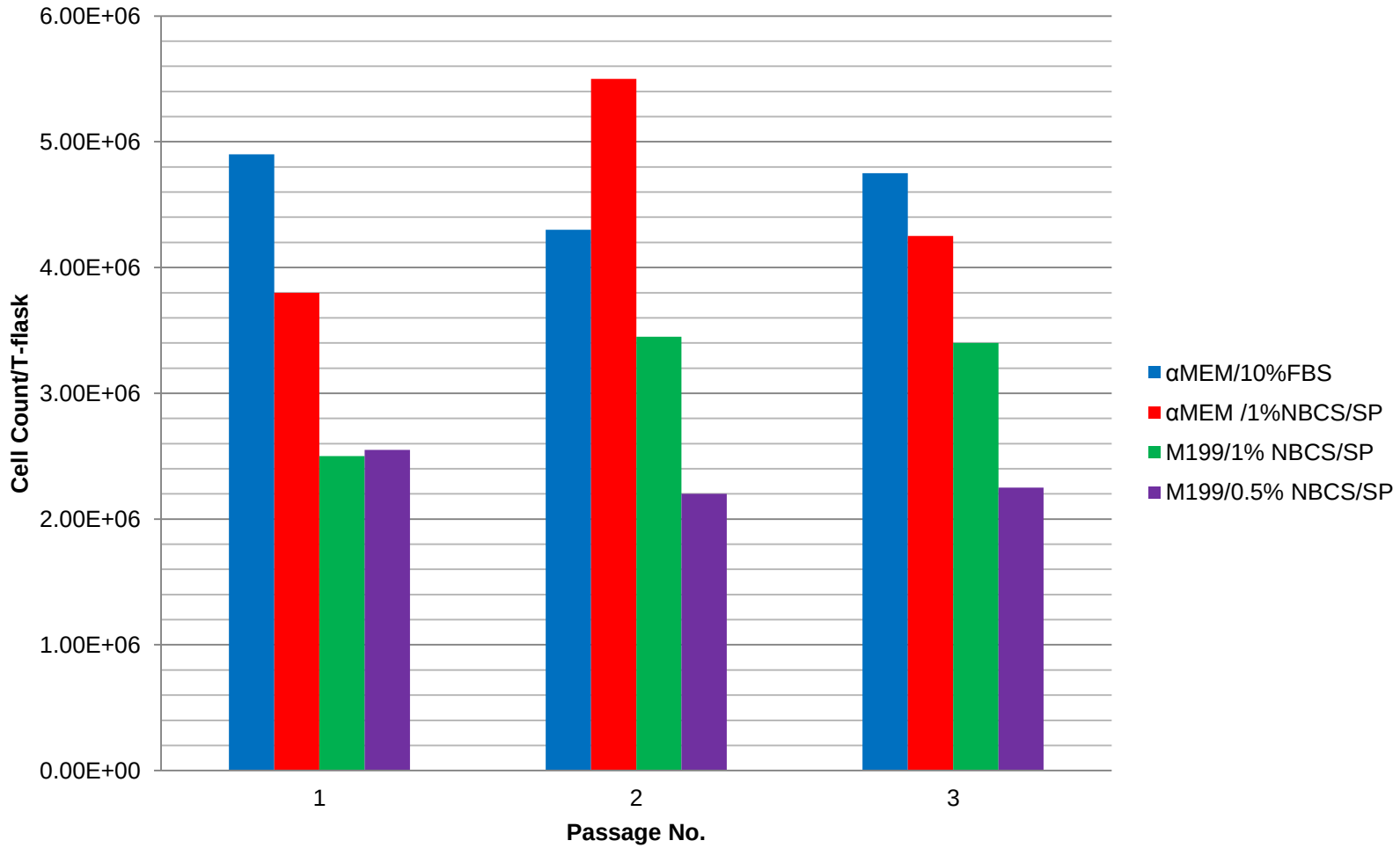
MDBK Cells


5.00E+06
4.50E+06
4.00E+06
3.50E+06
3.00E+06
2.50E+06
2.00E+06
1.50E+06
1.00E+06
5.00E+05
0.00E+00
Cell Count/T-flask
1
2
3
Passage No.
EMEM/10%FBS
EMEM /1%NBCS/SP
M199/1% NBCS/SP
M199/0.5% NBCS/SP

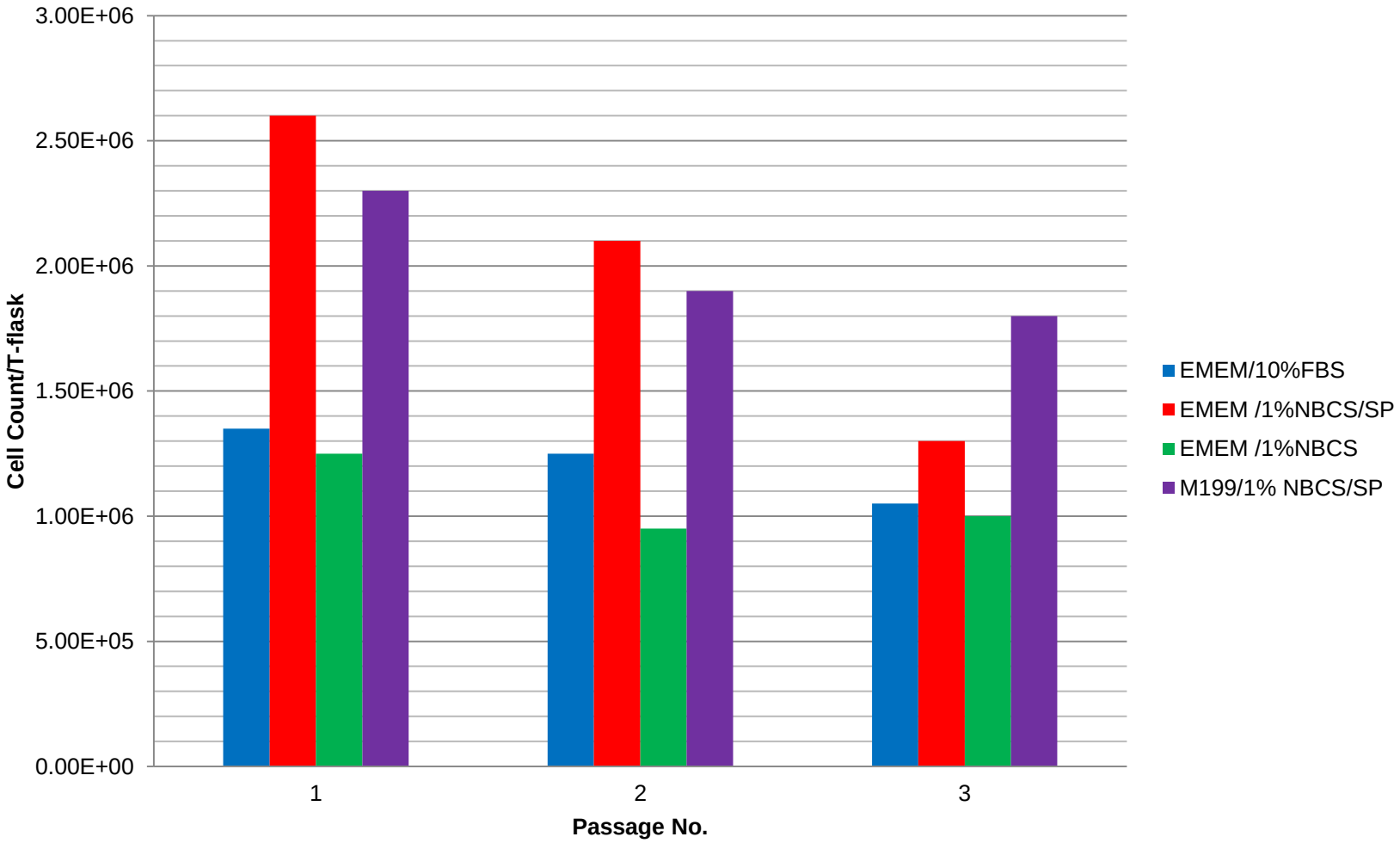
HEK293 Cells



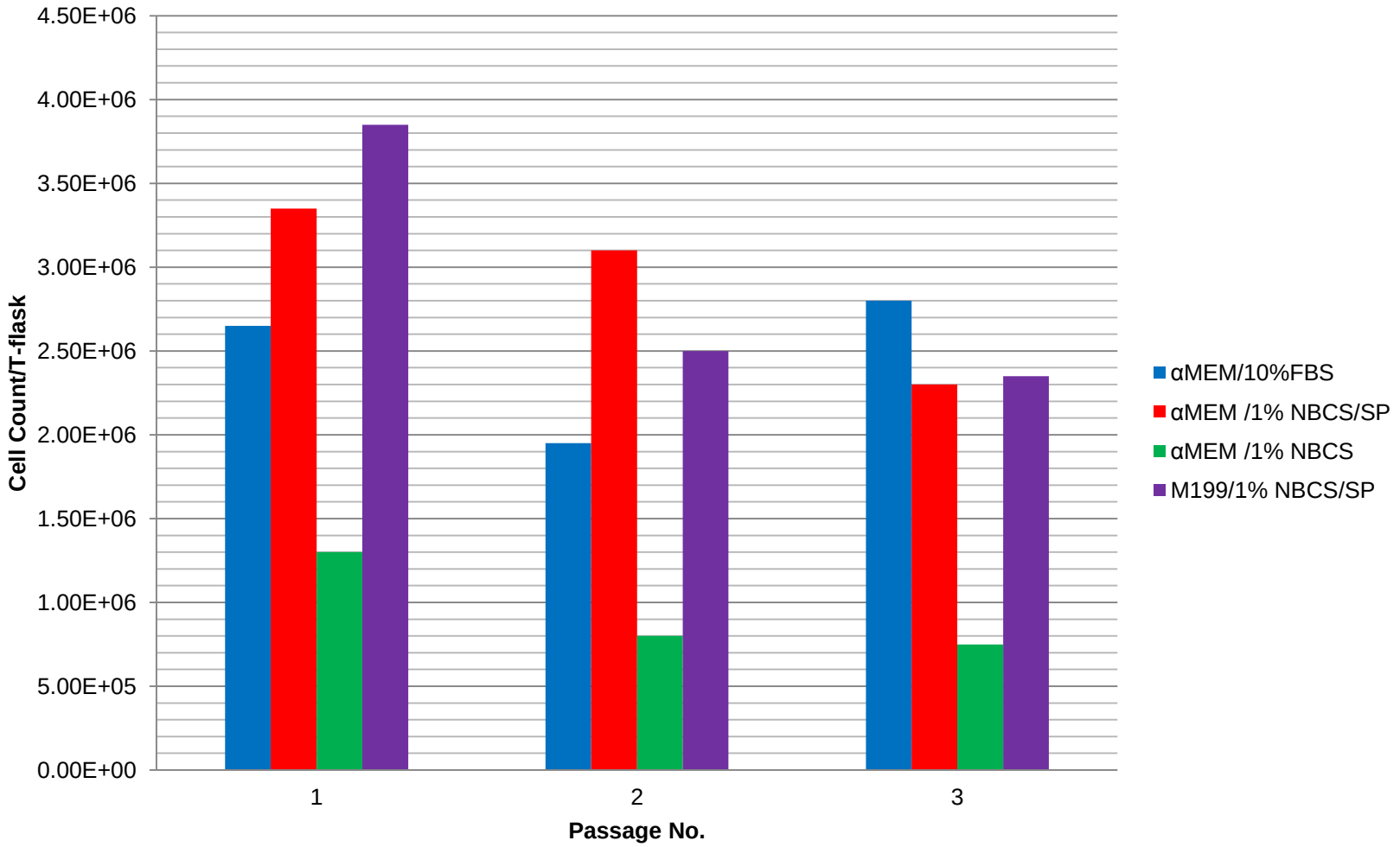
BHK21 Cells


6.00E+06
5.00E+06
4.00E+06
3.00E+06
2.00E+06
1.00E+06
0.00E+00
Cell Count/T-flask
1
2
3
Passage No.
αMEM/10%FBS
αMEM /1%NBCS/SP
M199/1% NBCS/SP
M199/0.5% NBCS/SP

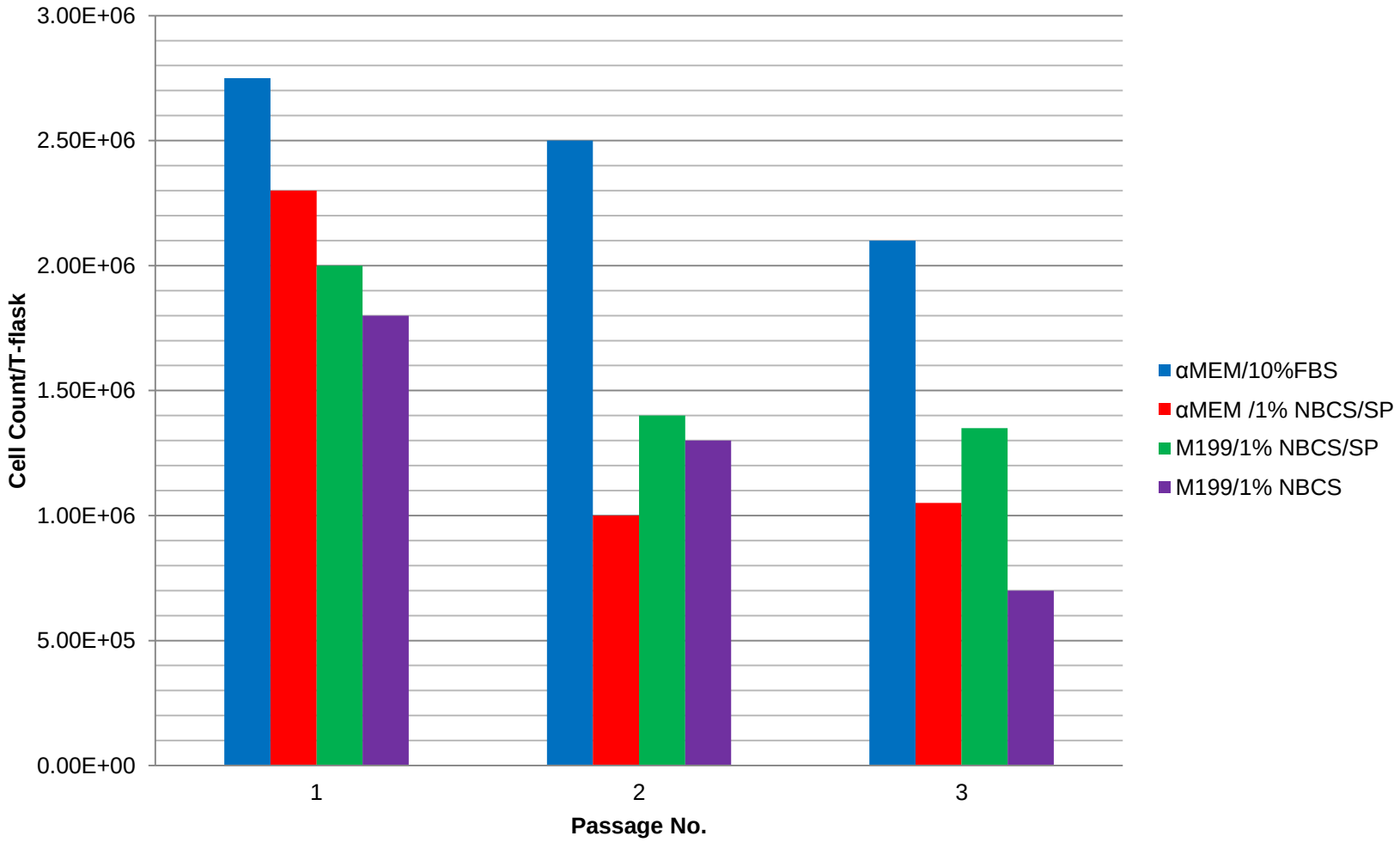
PK15 Cells


3.00E+06
2.50E+06
2.00E+06
1.50E+06
1.00E+06
5.00E+05
0.00E+00
Cell Count/T-flask
1
2
3
Passage No.
EMEM/10%FBS
EMEM /1%NBCS/SP
EMEM /1%NBCS
M199/1% NBCS/SP

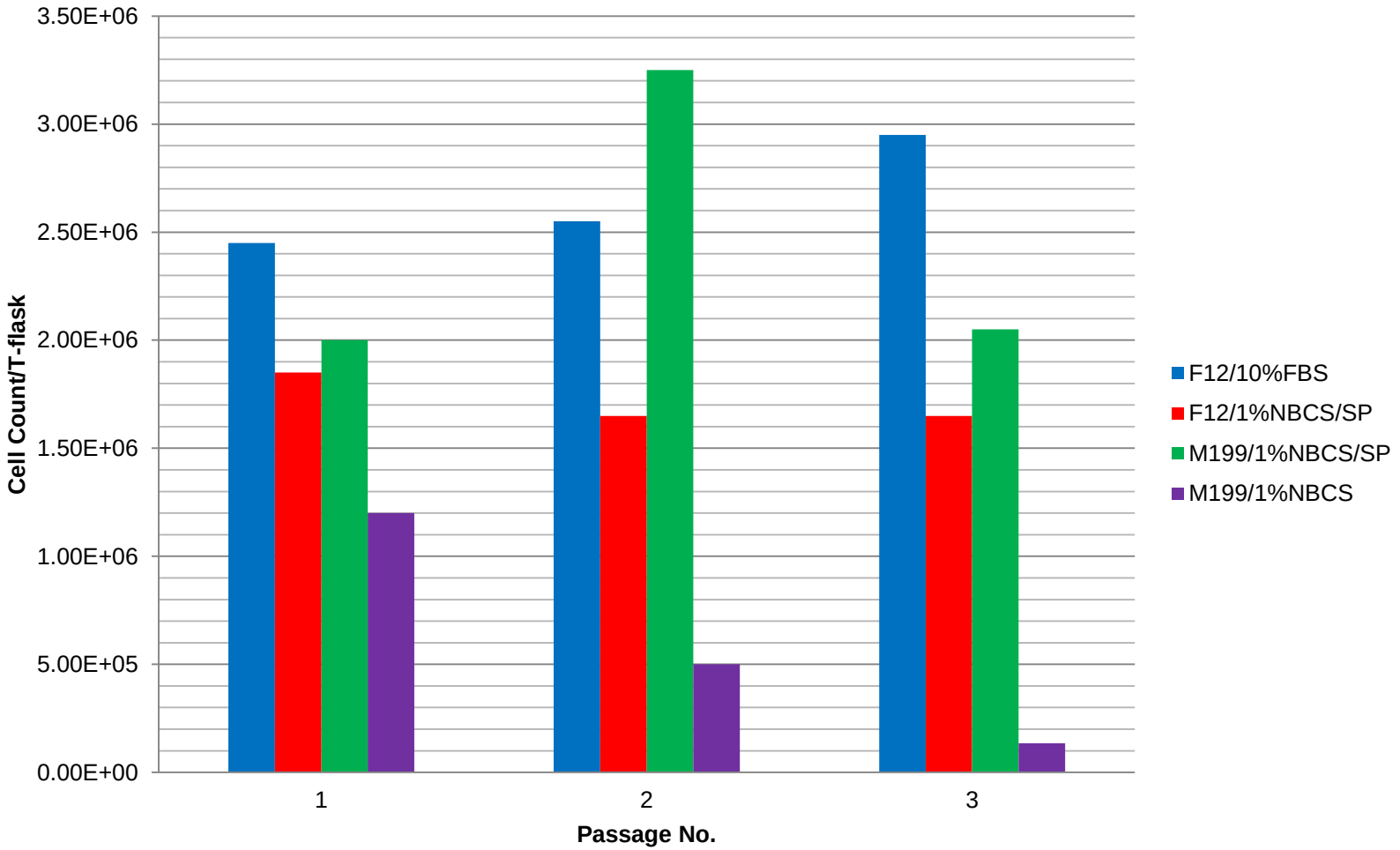
ST Cells


4.50E+06
4.00E+06
3.50E+06
3.00E+06
2.50E+06
2.00E+06
1.50E+06
1.00E+06
5.00E+05
0.00E+00
Cell Count/T-flask
1
2
3
Passage No.
αMEM/10%FBS
αMEM /1% NBCS/SP
αMEM /1% NBCS
M199/1% NBCS/SP

Marc 145 Cells



CHO Cells


3.50E+06
3.00E+06
2.50E+06
2.00E+06
1.50E+06
1.00E+06
5.00E+05
0.00E+00
Cell Count/T-flask
1
2
3
Passage No.
F12/10%FBS
F12/1%NBCS/SP
M199/1%NBCS/SP
M199/1%NBCS

Conclusion

1. Test result demonstrates that Super Plus supports most common used cell lines to replace 80% to 90% serum consumption with even higher cell growth rate than by using fetal bovine serum.
2. Reduced serum may cause loosely attachment issue in some cell lines such as HEK293, and CHO. Culture has to avoid vigorous shaking or agitation to avoid cell detachment during culture for those cell lines.
3. Most cell lines could be cultivated with their existed basal medium with reduced serum supply together with the Super Plus additives. Different basal media can be evaluated together in order to optimize the culture condition.