

Supplements

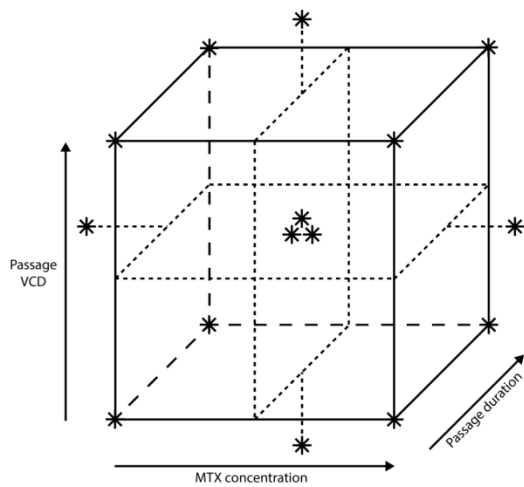


Figure S1. Schematic representation of the conducted central composite design for the Passage viable cell density, the MTX concentration and the passage duration. The eight corner points represent the -1/1 levels. The three stars in the middle of the cube represent the replicate centre-point runs (0 level). The outer stars represent the -2/2 levels for two of the three parameters.

Table S1. Risk assessment and FMEA results.

Process Parameter	Probability	Severity	Detection	RPN
MTX concentration	1	5	4	20
Passage duration	3	3	2	18
Passage VCD	2	2	4	16
pH	2	3	2	12
Thaw temperature	2	3	2	12
Thaw duration	2	2	3	12
Osmolality	1	3	3	9
Media storage temperature	1	4	2	8
Media storage time	1	4	2	8
Initial VCD	1	2	4	8
Type of cryo conservation	1	3	2	6
Media temperature	1	3	2	6
Cell line	1	5	1	5
Temperature	1	5	1	5
Incubator CO ₂	1	4	1	4
Agitation speed	1	4	1	4
Incubator humidity	1	3	1	3
Non-/baffled flasks	1	3	1	3
Working volume	1	2	1	2

Table S2. Numerical coding of varied process parameters.

	-2	-1	0	1	2
MTX concentration [nM]	0	7.5	15	22.5	30
Passage duration [d]	-	2	3,2	5	-
Passage VCD [x10⁶ cells/ml]	0.1	0.15	0.2	0.25	0.3

Table S3. Experimental set up and responses for the DoE.

Experiment number	MTX Concentration	Passage Duration	Passage VCD	Inoculum Viability [%]	Inoculum Growth Rate [1/d]	Production Viability [%]	Production Growth Rate [1/d]	Total mAb Titer [g/l]	Specific mAb Titer [g/l*(10 ⁶ cells*d/ml) ⁻¹]
1	-1	-1	-1	98.5	0.96	98.6	0.57	1.10	14.78
2	1	-1	-1	98.6	0.92	98.1	0.56	1.12	16.41
3	-1	1	-1	97.9	0.75	97.1	0.55	1.06	16.57
4	1	1	-1	98.0	0.79	97.3	0.51	1.04	17.32
5	-1	-1	1	98.7	0.86	98.7	0.56	1.11	17.26
6	1	-1	1	98.7	0.87	98.0	0.55	1.09	16.47
7	-1	1	1	97.4	0.69	97.3	0.49	0.98	20.87
8	1	1	1	96.6	0.68	96.6	0.44	0.82	23.30
9	-2	0	0	98.8	0.90	98.7	0.57	1.19	16.72
10	2	0	0	98.4	0.82	98.2	0.52	1.01	19.03
11	0	0	-2	98.2	0.79	97.4	0.48	0.99	20.21
12	0	0	2	97.9	0.72	97.4	0.48	0.90	19.58
13	0	0	0	98.9	0.89	98.3	0.55	1.16	17.12
14	0	0	0	98.5	0.88	98.2	0.57	1.06	16.11
15	0	0	0	98.8	0.87	98.6	0.55	1.08	17.08

Table S4. Model statistics for the inoculum expansion and production process responses

	R ²	R ² _{adj}	Q ²	p-value	Reproducibility
Inoculum viability	0.95	0.91	0.79	0.0001	0.90
Inoculum growth rate	0.99	0.98	0.96	< 0.0001	0.99
Production viability	0.89	0.81	0.62	0.0019	0.87
Production growth rate	0.99	0.90	0.69	< 0.0001	0.92
Total mAb titer	0.93	0.88	0.70	0.0003	0.71
Specific mAb titer	0.94	0.914	0.827	< 0.0001	0.90