Current Approaches in Cell Culture Fluid Harvesting

Cell Culture

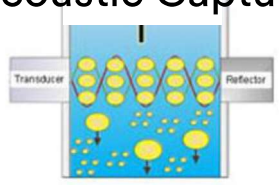
Primary Cell Mass Separator Stage

Depth Filter

Centrifuge

Body Feed

Acoustic Capture

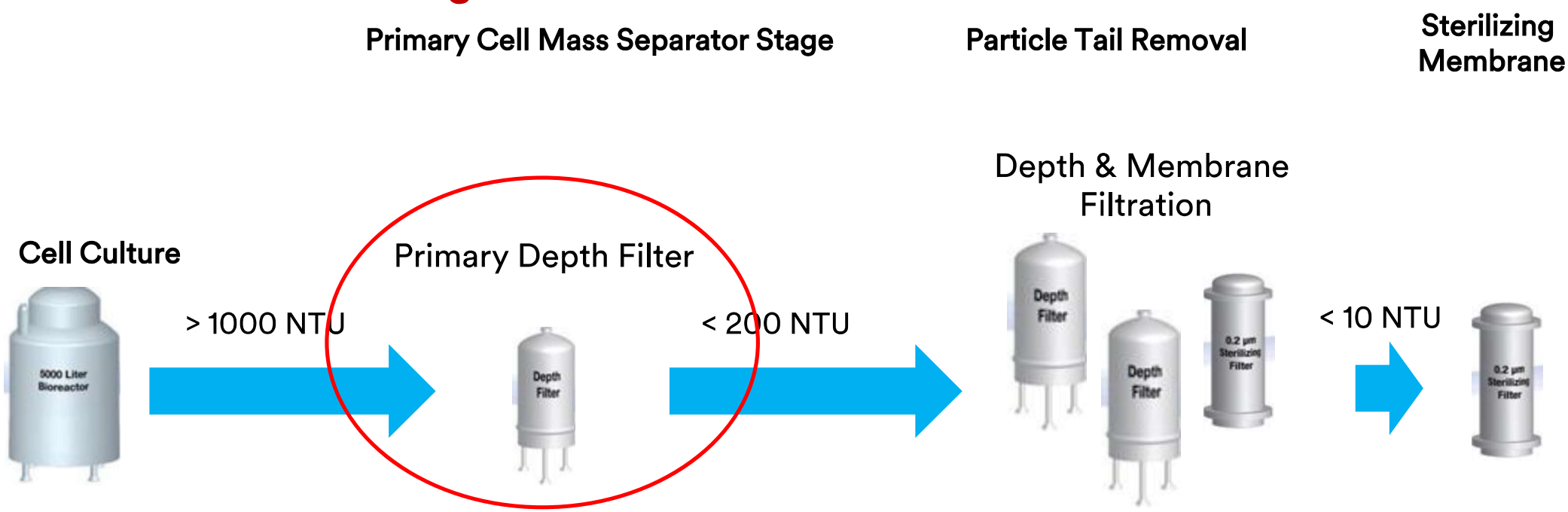


Particle Tail Removal

Depth & Membrane Filtration

Sterilizing Membrane

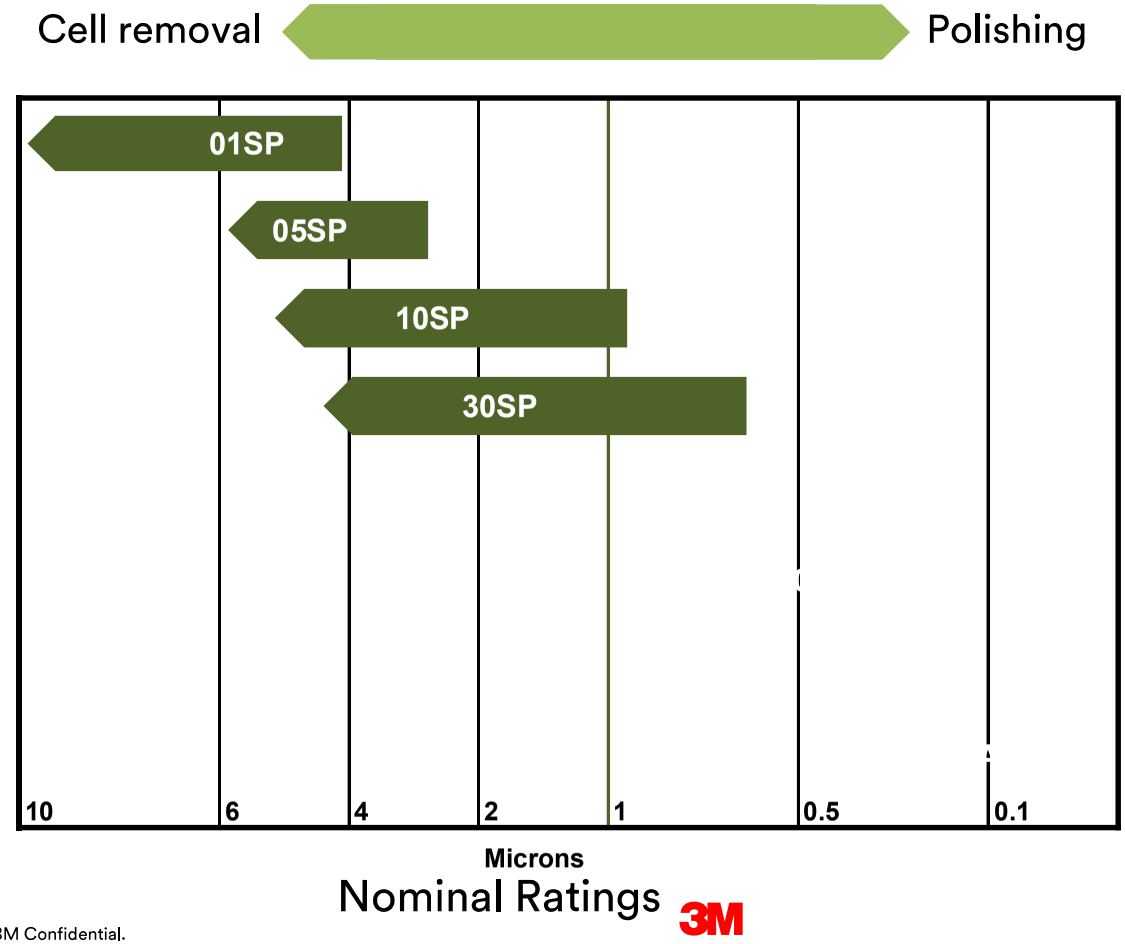
Clarification Using Filtration



- Cell capture
- Large cell debris capture

3M

3M Zeta Plus™ Primary Clarification Depth Filtration Grades



Testing Primary Harvesting Grades for Cell Shear

mAb CHO Culture
~ 6 * 10^6 cell/cm^3
99 % viability

~ 120 LMH
< 15 psid
< 100 NTU

- Turbidity
- Dynamic light scattering (< 4 um)
- Coulter counter (4- 34 um)

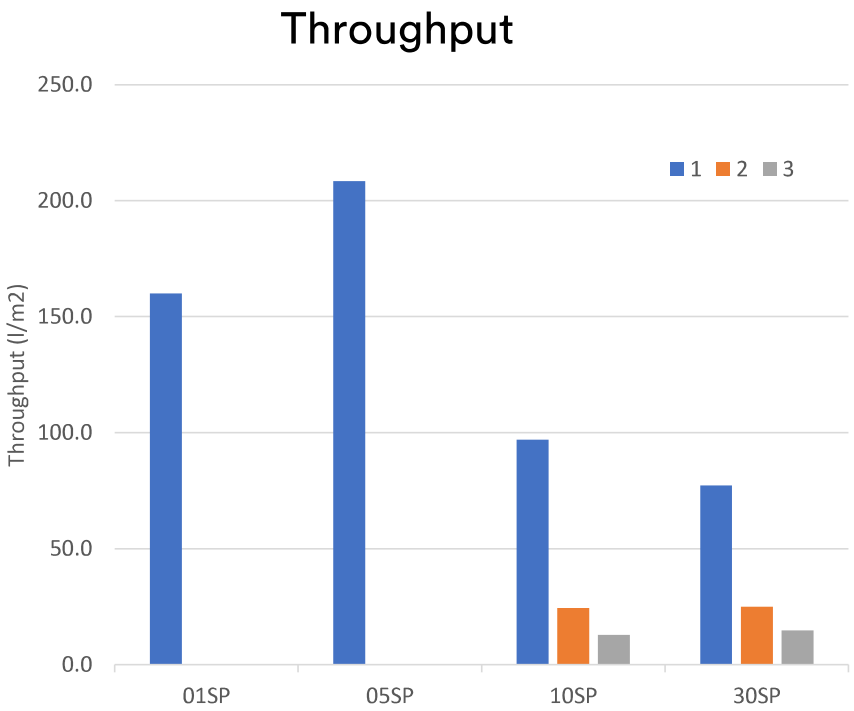
0 – 5 psid
Pool 1

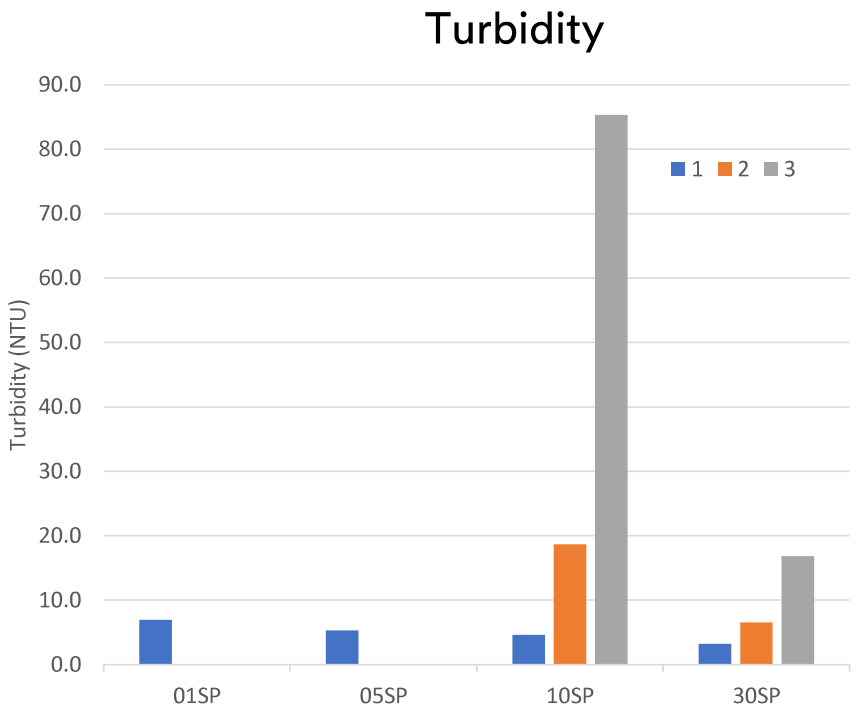
5 – 10 psid
Pool 2

10 – 15 psid
Pool 3

3M

Throughput & Turbidity

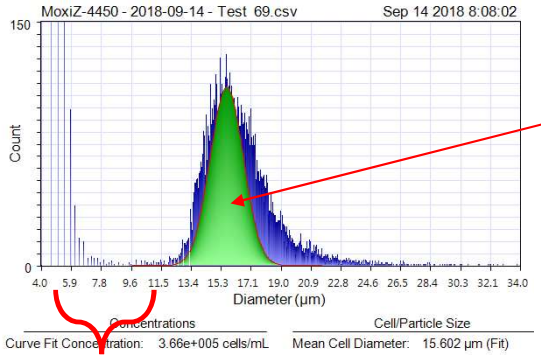




1 – 0-5psi
2 – 5-10psi
3 – 10-15psi

Coulter Counter Analysis (4 – 34 µm) at 0-5 psid

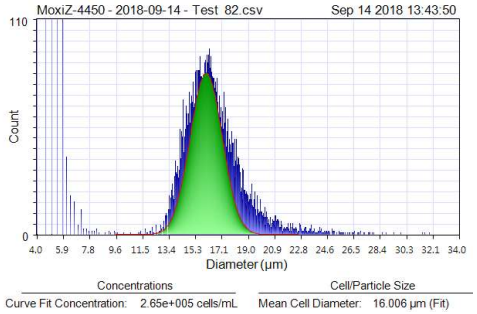
Feed (99% viability)



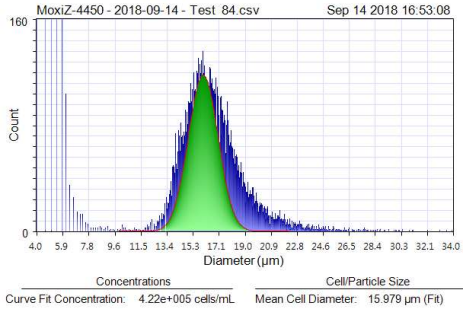
Viable Cells

Shear Field

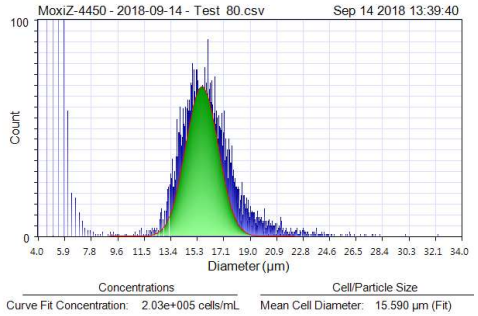
01SP – 1



05SP – 1

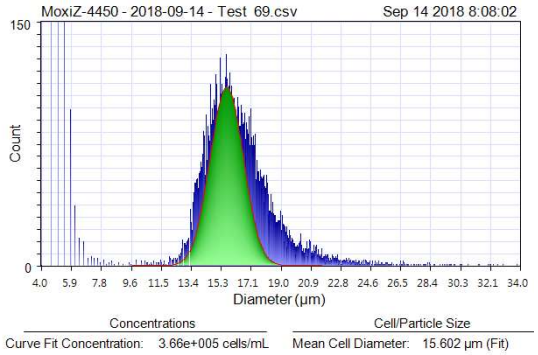


10SP – 1



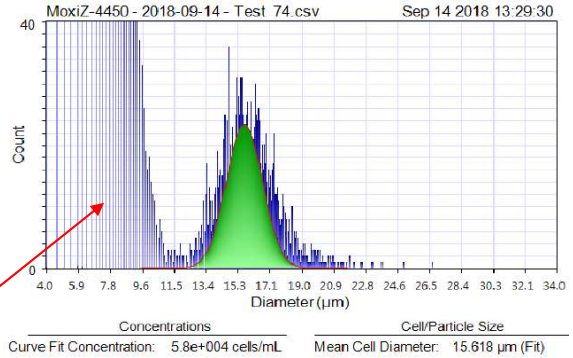
Coulter Counter Analysis (4 – 34 µm) at 5-15 psid

Feed (99% viability)

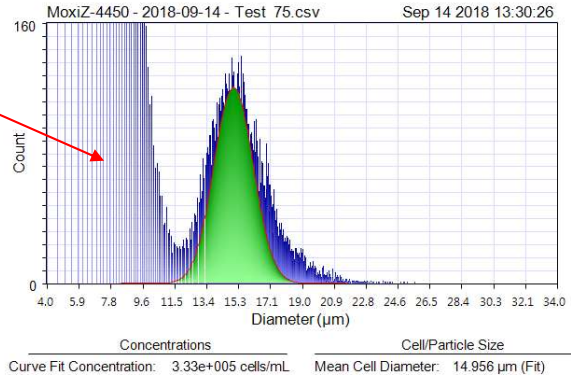


Shear field

10SP – 2

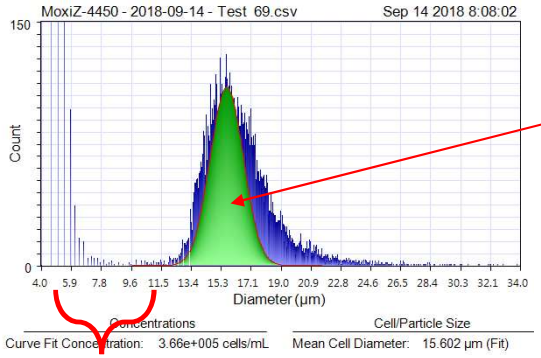


10SP – 3



Cell Shear in 10SP Zeta Plus Grade

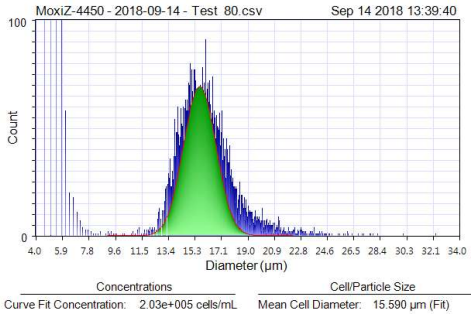
Feed (99% viability)



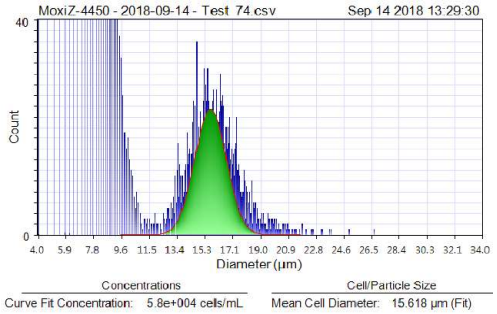
Viable Cells

Shear Field

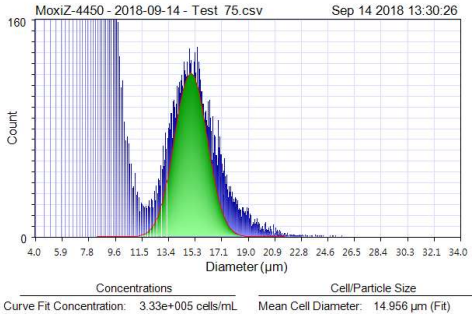
0-5 psid



5-10 psid



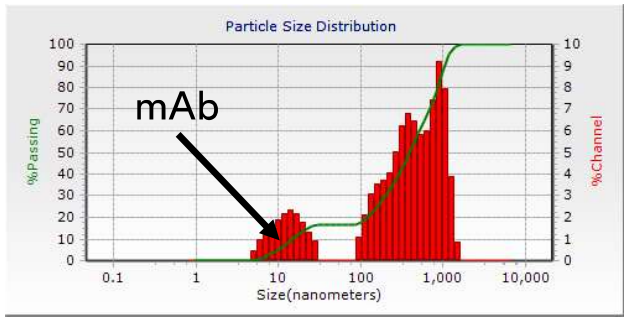
10-15 psid



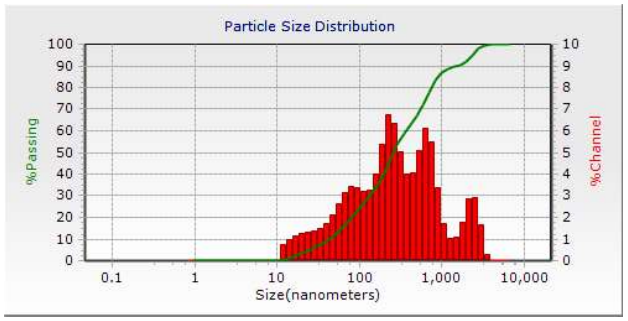
Dynamic Light Scattering (DLS) of 10SP Filtrate

- Shear produces a lot of particles all over the size spectrum

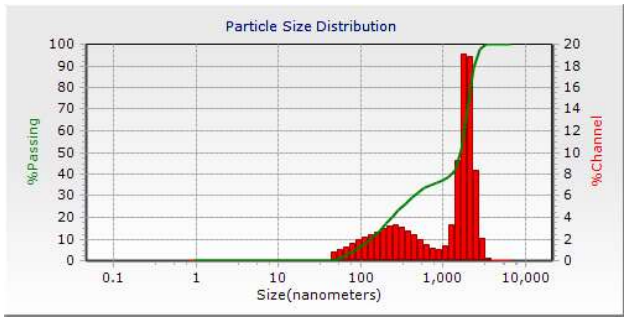
0-5 psid



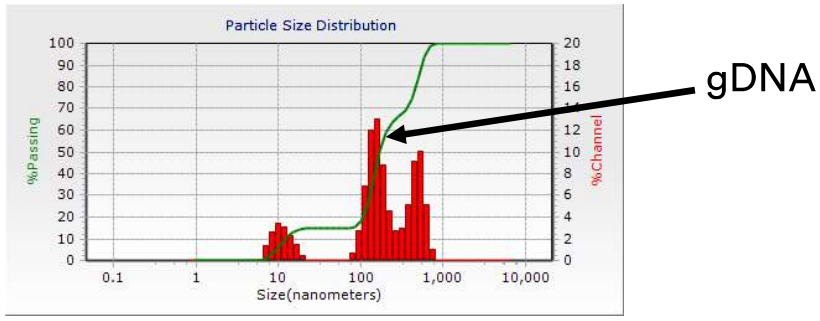
5-10 psid



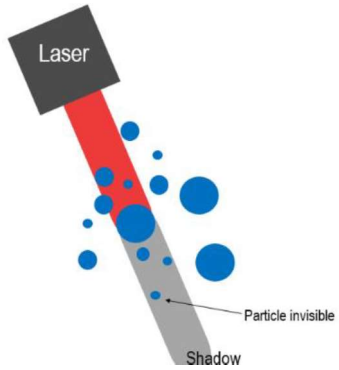
10-15 psid



05SP DLS at ~180 l/m²



DLS Technique

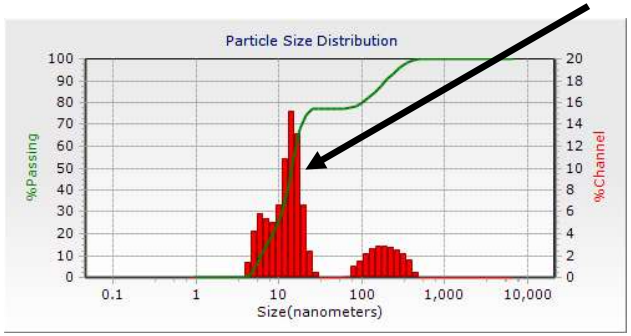


Dynamic Light Scattering (DLS) of 30SP Filtrate

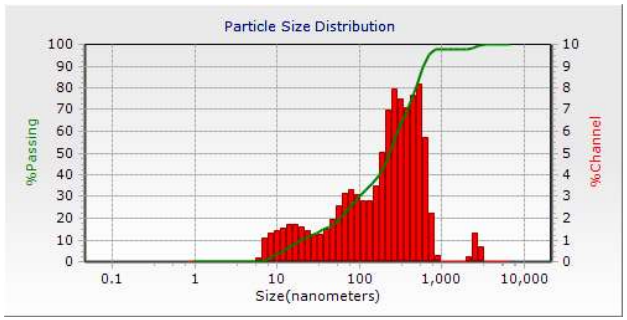
- Cells being extruded through the filter pores under pressure

0-10 psid

mAb

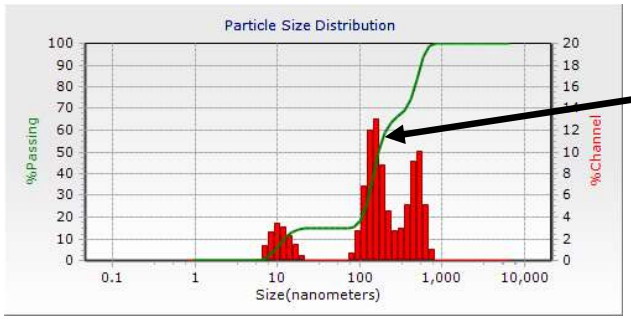


10-15 psid (~ 120 l/m^2)

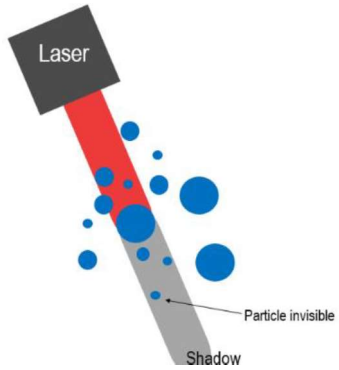


05SP DLS at ~180 l/m^2

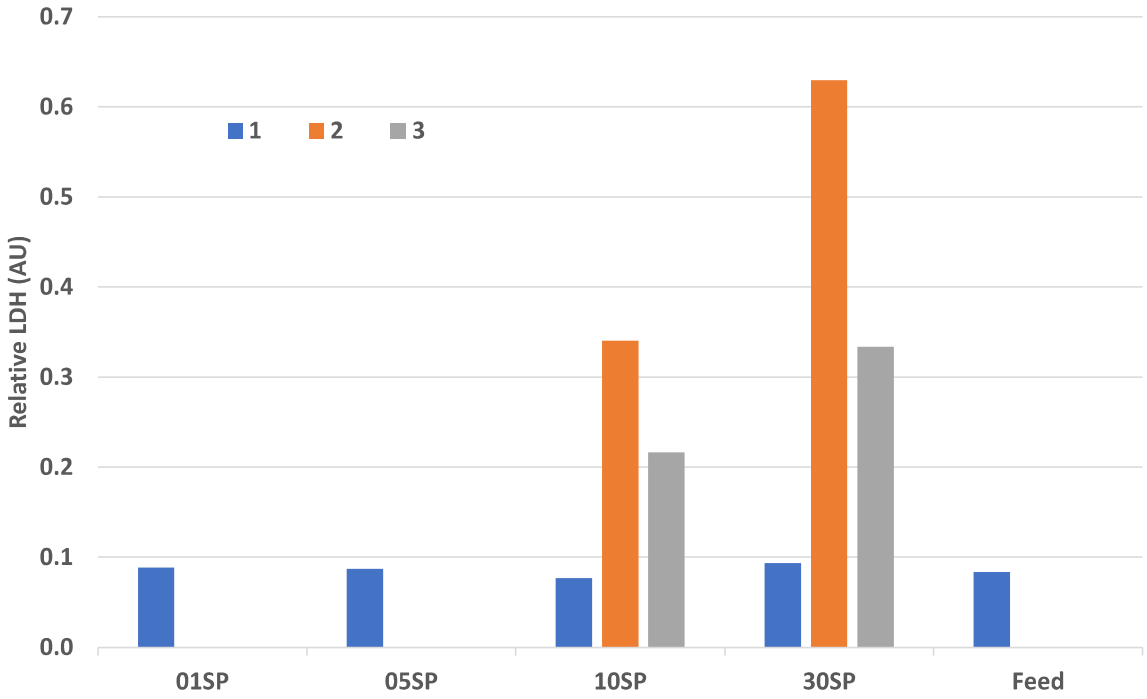
gDNA



DLS Technique



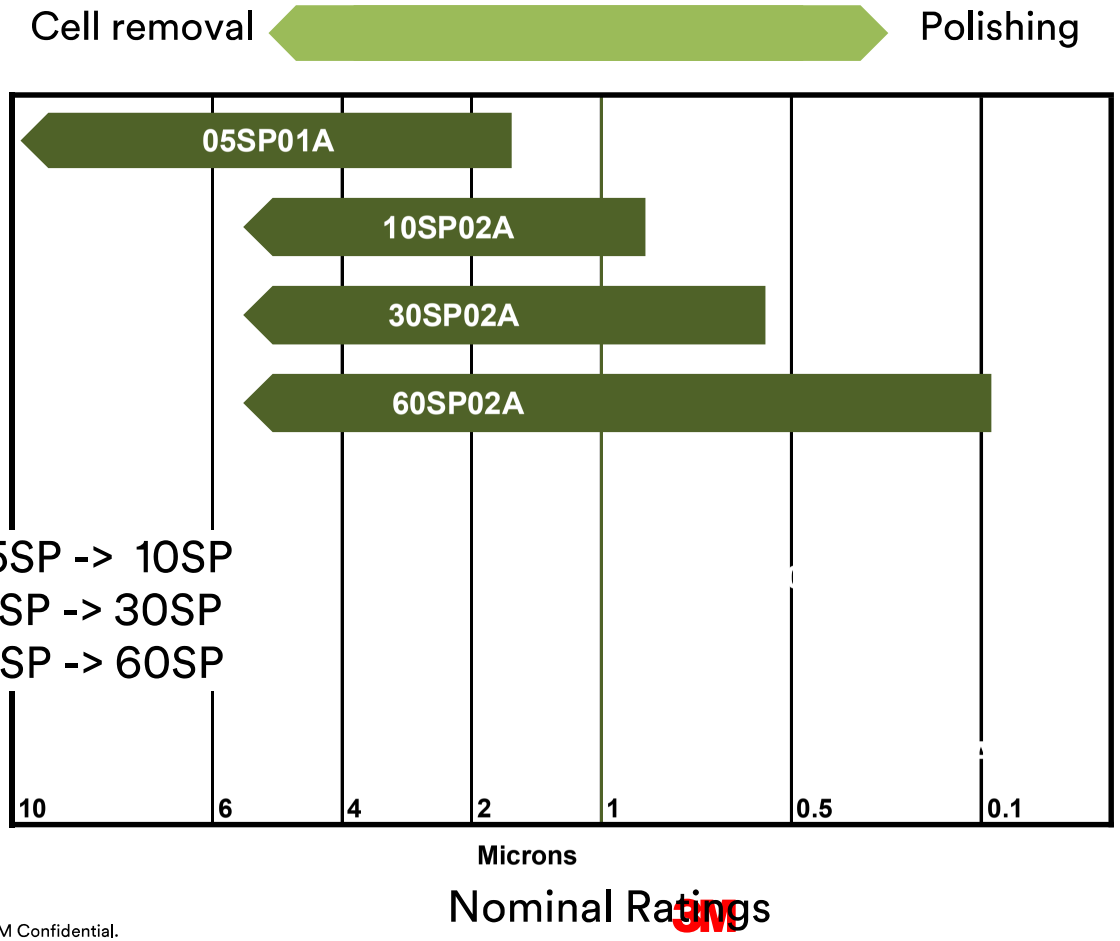
Lactate Dehydrogenase (LDH) Assay



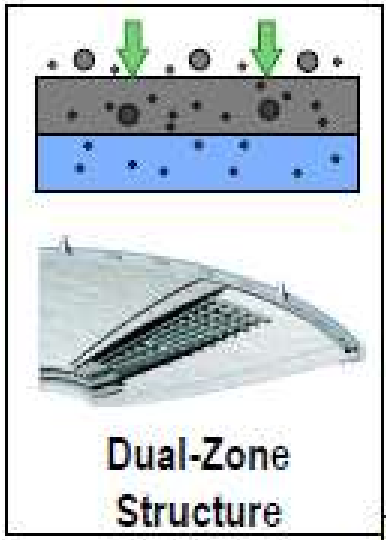
- Why is LDH lower in fraction 3 compared to that in fraction 2?

3M

Dual Layer Zeta Plus Products – Deep Funnel for High Clarification Performance



SP – low charge media



- 10SP02 = 05SP -> 10SP
- 30SP02 = 05SP -> 30SP
- 60SP02 = 05SP -> 60SP

Shear Dynamics in Dual Layer Harvest Grade Zeta Plus Encapsulated Products

mAb CHO Culture
~ 8 * 10^6 cell/cm^3
98 % viability

- Depth Filter

~ 120 LMH
< 15 psid
< 100 NTU

Terminal Event:
Pressure P1
< 11 psid

Particle Sizing Sample

- 0.2 µm PES membrane
~300 LMH

Terminal Event:
Pressure P2
< 5 psid
(turbidity/
break through trap)

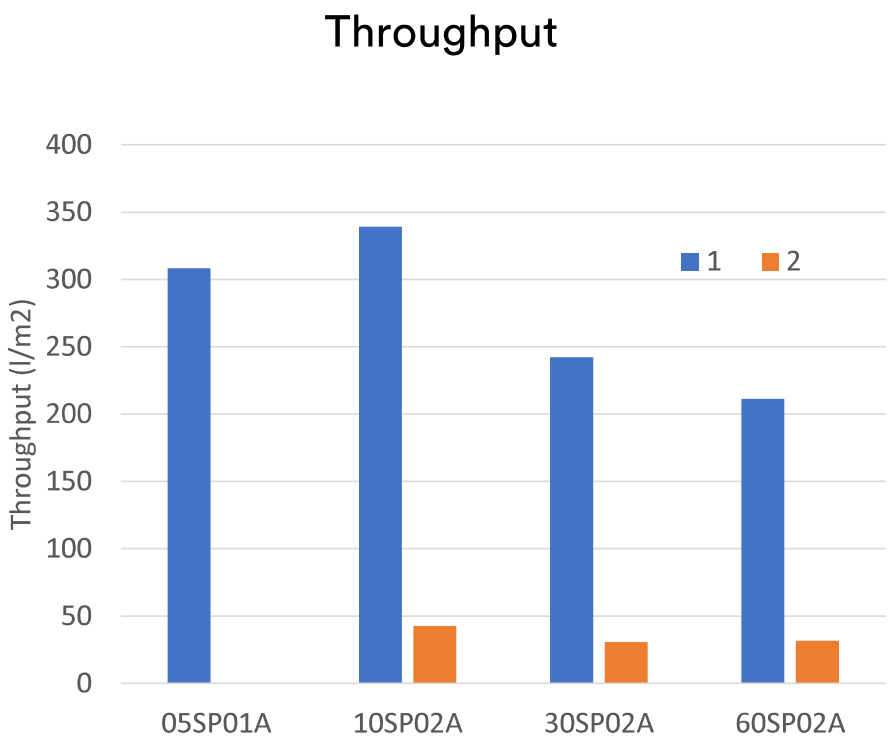
Pool 1
P1 = 0-6 psid)

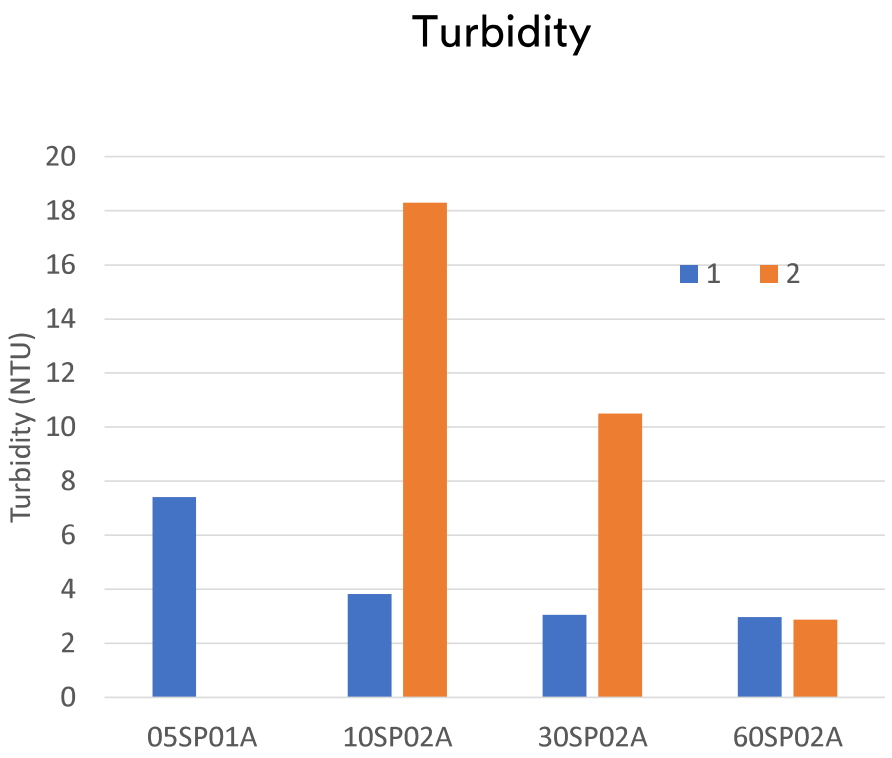
Pool 2
P1 = 6-16 psi)

- Turbidity
- Dynamic light scattering (< 4 um)
- Coulter counter (4- 34 um)
- Protein gel product quality analysis

3M

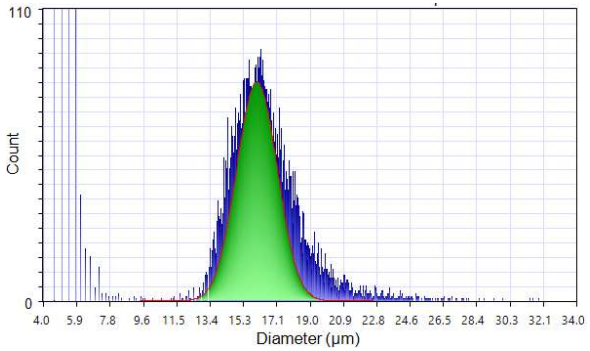
Throughput & Turbidity



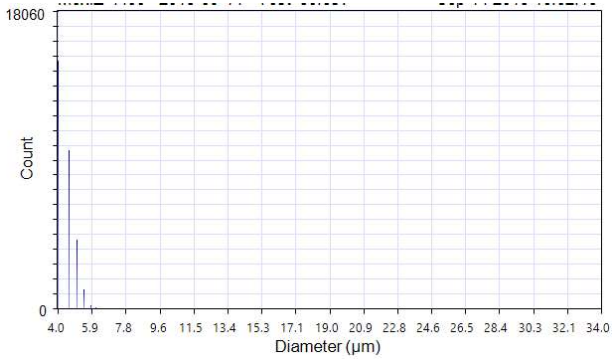


Clarification Dynamics of 05SP01A

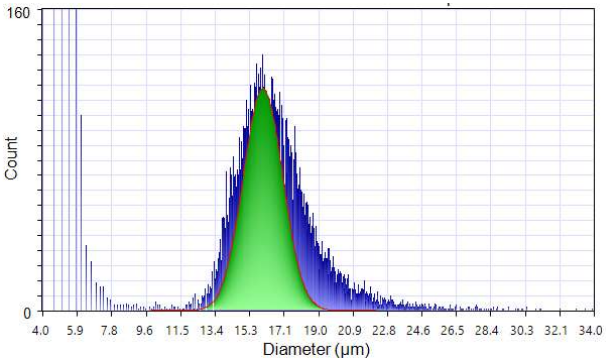
Feed
98% viability



05SP01A
P1 <= 3 psid



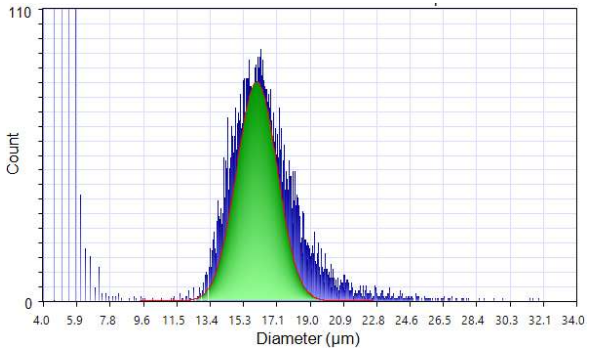
05SP01A
Terminal Event
Breakthrough: P2 = 5 psid



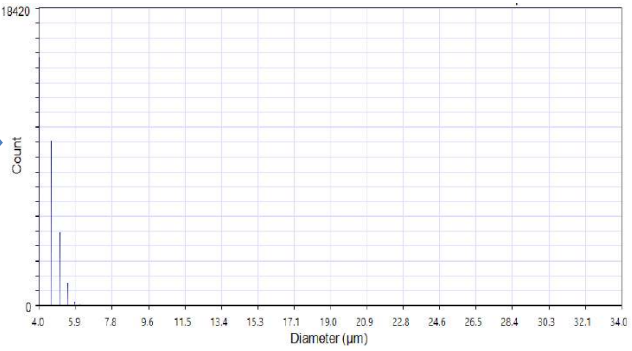
- 3M ZetaPlus™ 05SP01A Breaks through at ~ 3 psi with almost no cell shear

Clarification Dynamics of 10SP02A

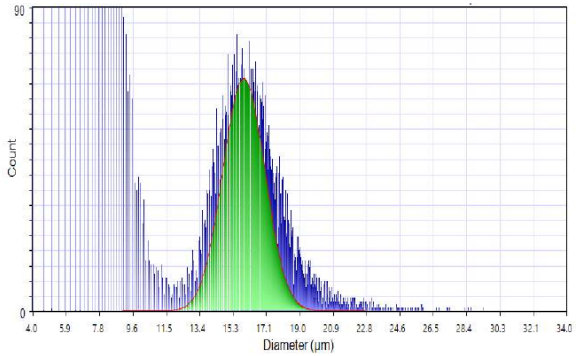
Feed
98% viability



10SP02A
Terminal event : P1 = 6 psid



10SP02A
Terminal Event: P2 = 5 psid
(P1 = 11 psid)

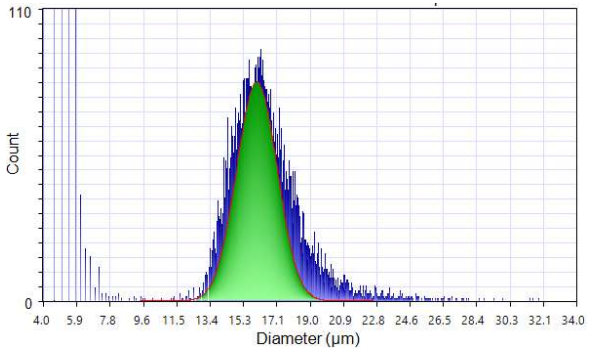


- 3M ZetaPlus™ 10SP02A Pressures out
- Above ~ 6 psi, filter produced a lot of shear and turbidity, clogging the membrane

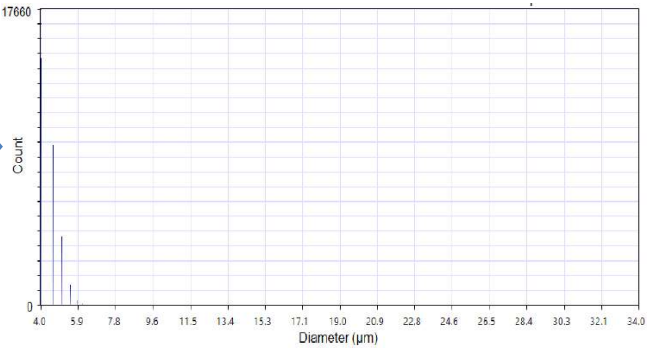
3M

Clarification Dynamics of 30SP02A

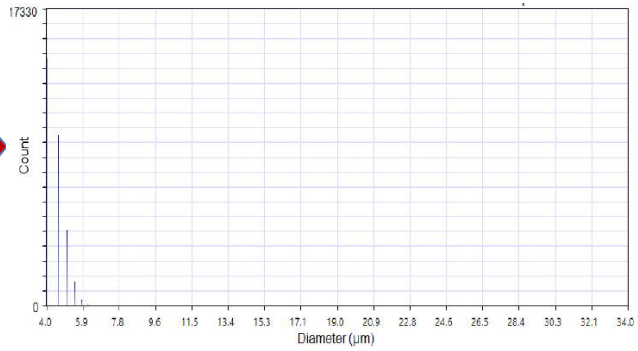
Feed
98% viability



30SP02A
Terminal event : P1 = 6 psid



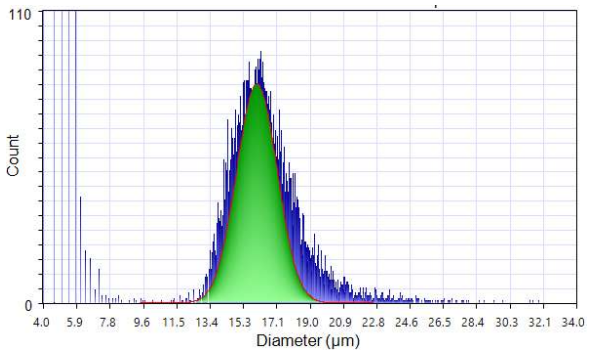
30SP02A
Terminal Event: P1 = 16 psid
(P2 < 2 psid)



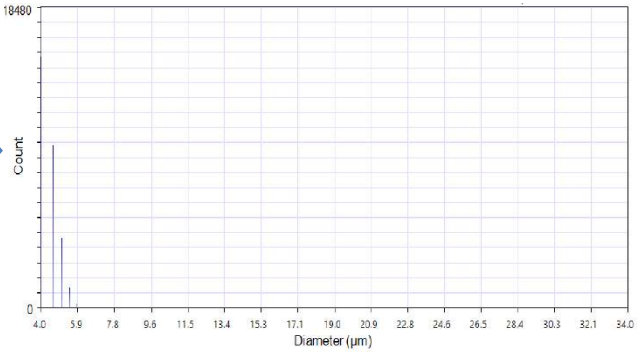
- 3M ZetaPlus™ 30SP02A Pressures out without any large (> 4 µm) cell fragments coming through

Clarification Dynamics of 60SP02A

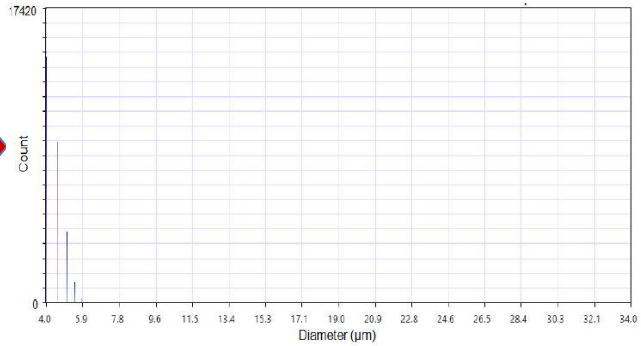
Feed
98% viability



60SP02A
Terminal event : P1 = 6 psid



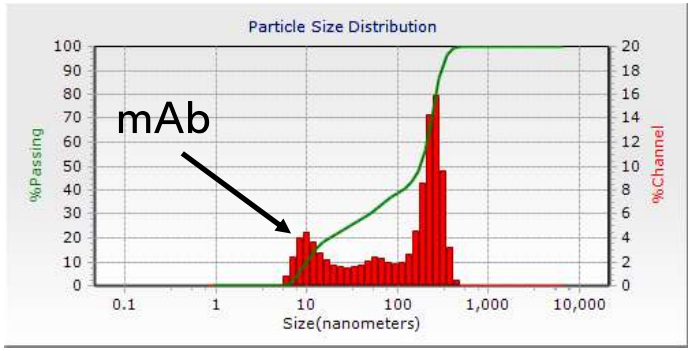
60SP02A
Terminal Event: P1 = 16 psid
(P2 < 2 psid)



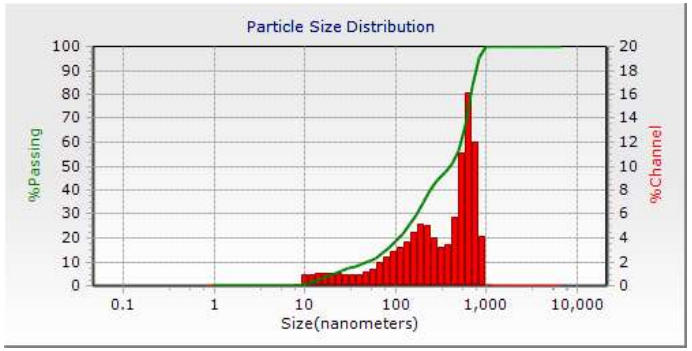
- 3M ZetaPlus™ 60SP02A Pressures out without any large (> 4 µm) cell fragments coming through

Observing Sub-micron Shear at Terminal Throughput

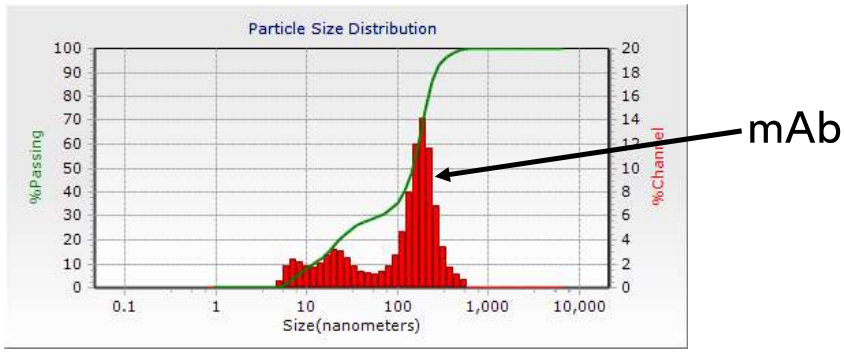
05SP01A (~ 3 psi)



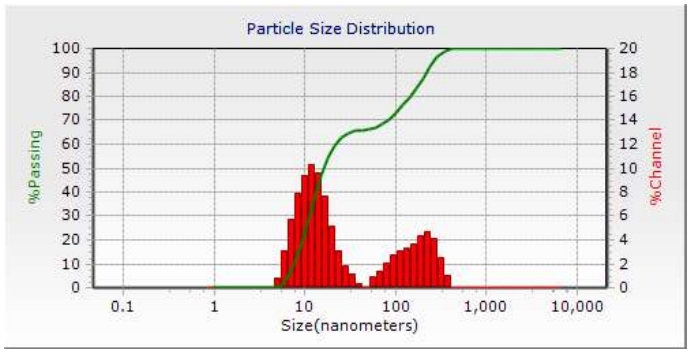
10SP02A (~ 11 psi)



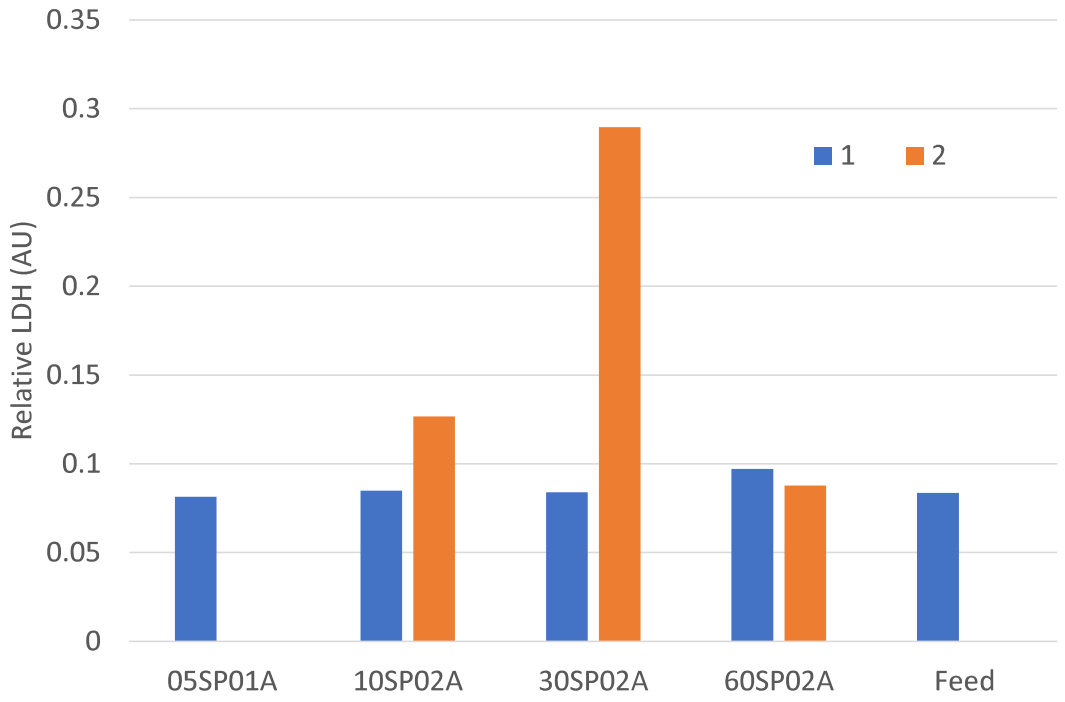
30SP02A (~ 16 psi)



60SP02A (~ 16 psi)



Lactate Dehydrogenase (LDH) Assay



- 10SP02A and 30SP02A produce a lot of shear and LDH activity in CCCF
- 60SP grade in 60SP02 is too tight for cell to come in and does not shear cells

Analyzing mAb Product Reduction Using Protein Gels

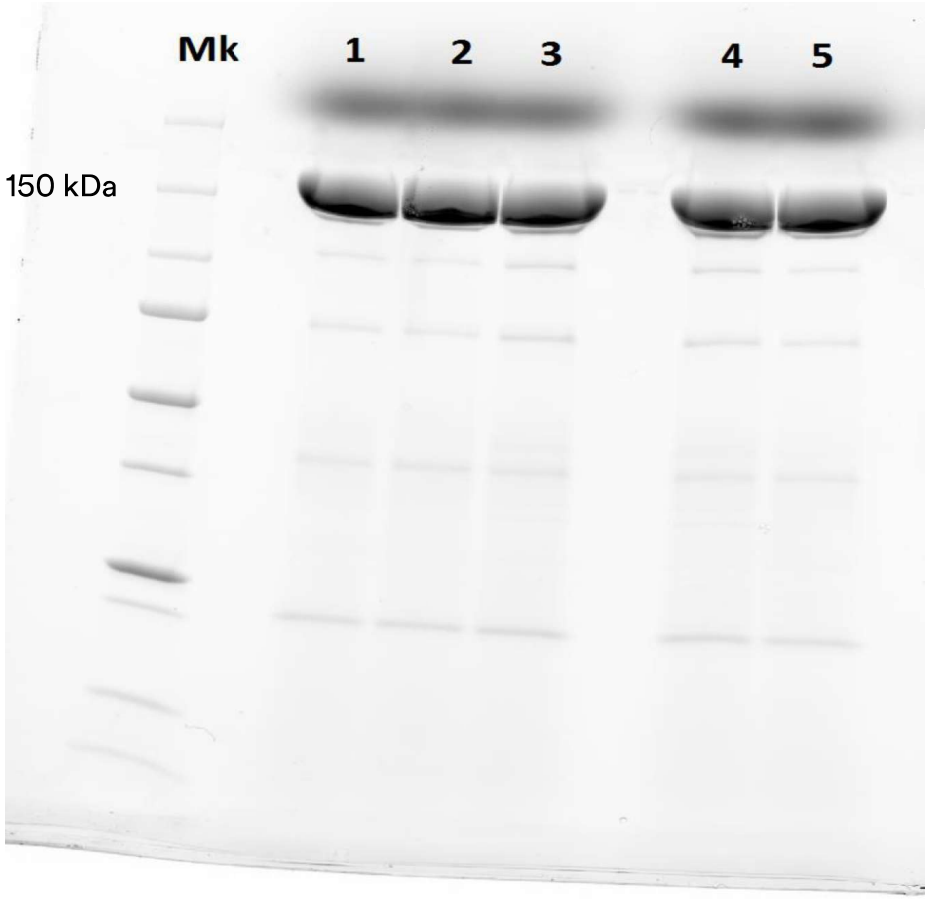
1 – Feed

2- 05SP01A

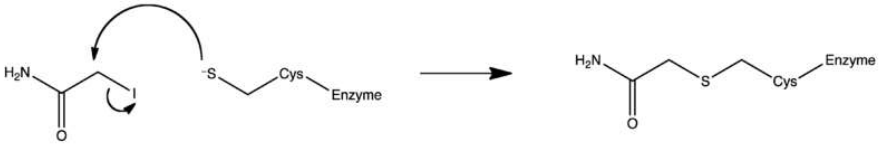
3- 10SP02A

4- 30SP02A

5- 60SP02A



Cap reduced sulfhydryl groups using Iodoacetamide



IAM Cys Capped Cys

Analyzing mAb Product Reduction Using Protein Gels

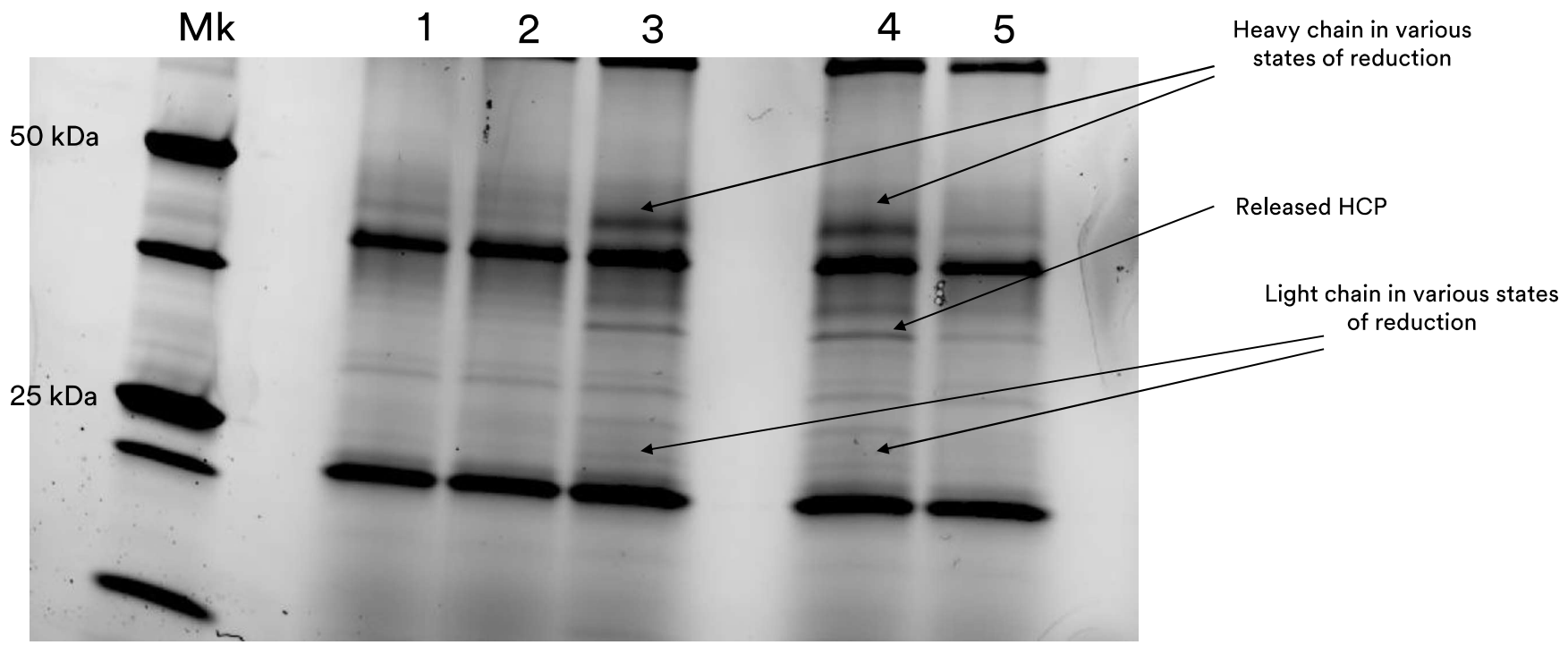
1 – Feed

2- 05SP01A

3- 10SP02A

4- 30SP02A

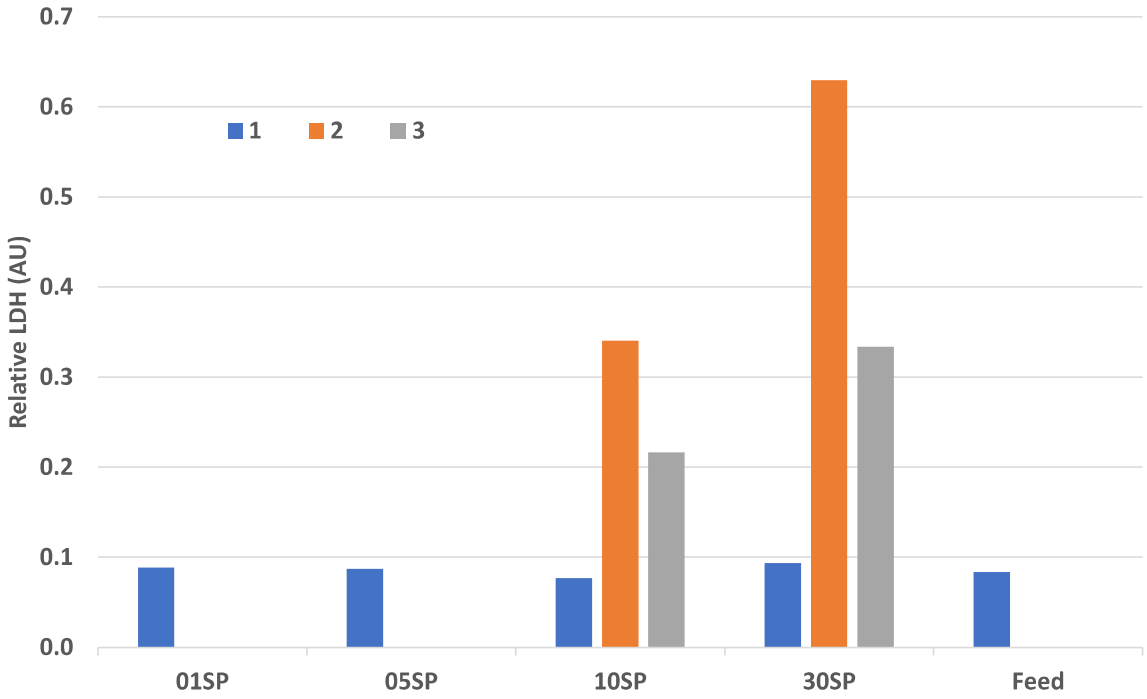
5- 60SP02A



- Shear in 10SP02A and 30SP02A grade filters releases reductases that degrade the product quality

3M

Lactate Dehydrogenase (LDH) Assay



- Why is LDH lower in fraction 3 compared to that in fraction 2?

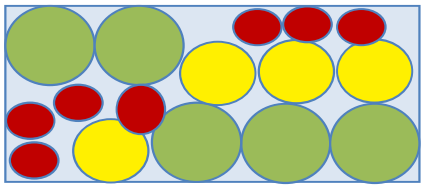
3M

Consider Depth Filter Loading Dynamics

Pore for gentle cell capture

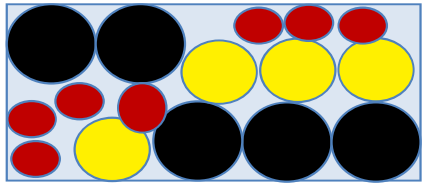
Pore that shears the cell

Pore too small for cell to enter



Low Shear

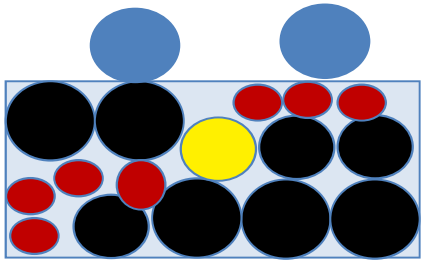
HCCF



0 – 5psid

High Shear

HCCF

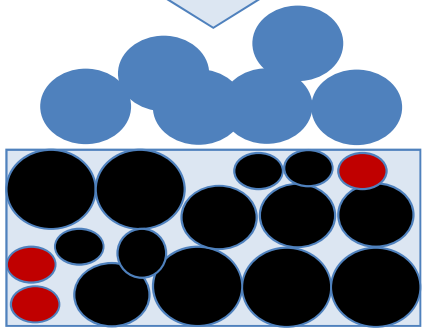


Cell fragments,
LDH, enzymes

5 – 10psid

Low Shear

HCCF



Pores too small for cells,
Filter pressures out

10 – 15psid

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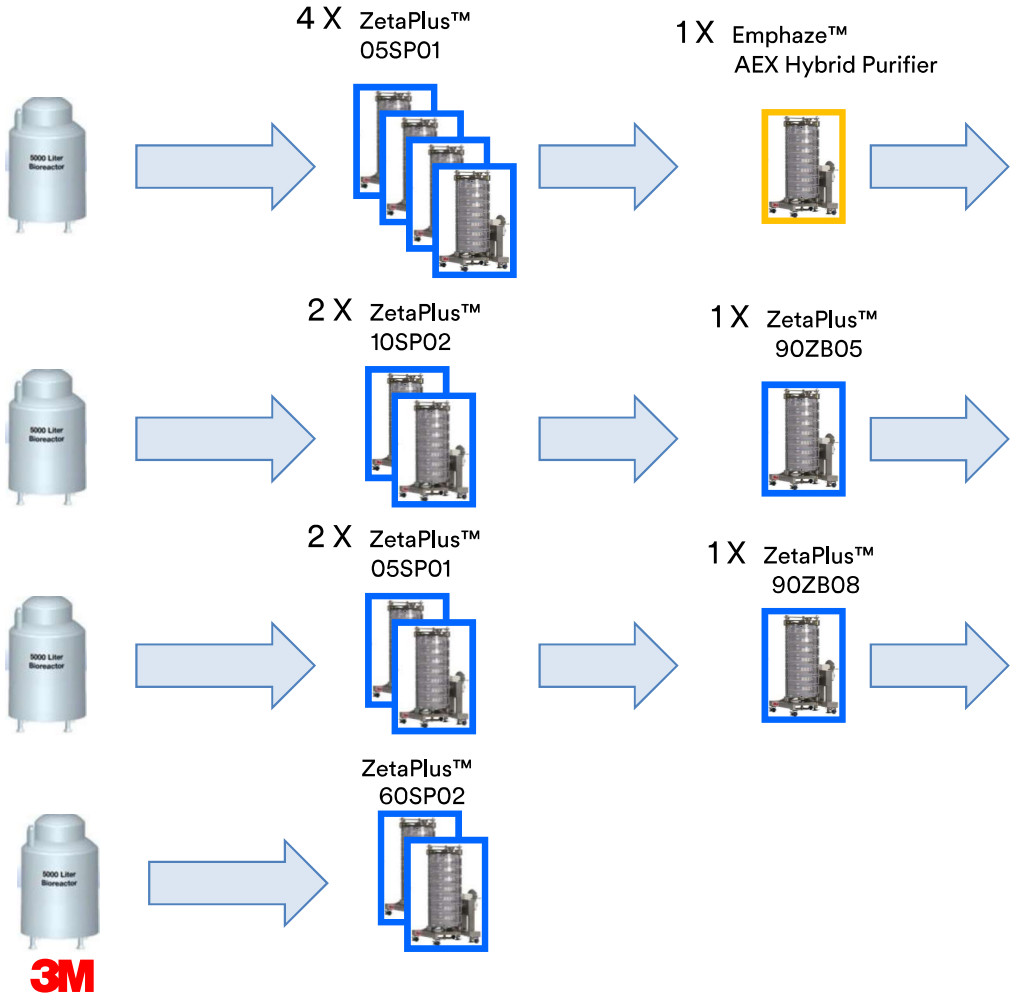
Clarification Train Design Using Different Strategies

- Train 1 – Low Shear Advanced
  - Small total footprint
  - DNA removal
  - Chromatographic clarification
- Train 2 – Typical Conventional
  - Conventional funnel strategy
  - Some charge on second stage
  - Demonstrates cell shear
- Train 3 – Typical Low Shear
  - Low shear funnel strategy
  - Some charge on second stage
  - Tries to avoid cell shear
- Train 4 – Conventional 1 stage
  - Singe stage clarification
  - Little to no charge
  - May have some shear



Depth Filter Grade Sequence in the Trains

- Train 1

4 X ZetaPlus™ 05SP01

01SP -> 05SP

1 X Emphaze™ AEX Hybrid Purifier

Q FNW -> 0.2 um membrane

- Train 2

2 X ZetaPlus™ 10SP02

05SP -> 10SP

1 X ZetaPlus™ 90ZB05

30ZB -> 90ZB

- Train 3

2 X ZetaPlus™ 05SP01

01SP -> 05SP

1 X ZetaPlus™ 90ZB08

60ZB -> 90ZB

- Train 4

ZetaPlus™ 60SP02

05SP -> 60SP

Low Shear
Mild Shear
High Shear
Dual mode

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Cell Shear in the Clarification Trains

Trains:

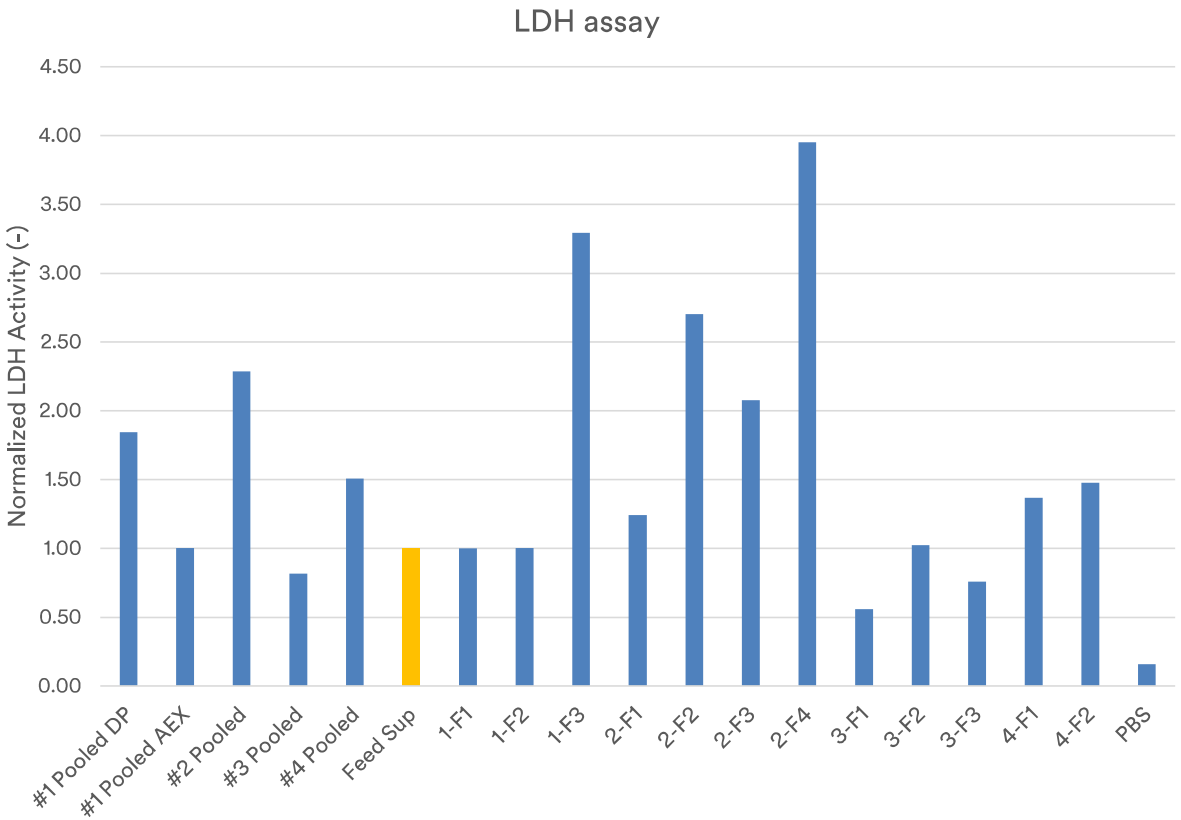
#1: 4X 05SP01 -> 1X Emphaze AEX HP
#2: 2X 10SP02 -> 1X 90ZB05
#3: 2X 05SP01 -> 1X 90ZB08
#4: 60SP02

Conditions:

Cell culture viability: ~ 80%
Flowrate: 100 LMH
End point: 15 psid on any stage

Data:

#1 Pooled DP – terminal pressure
#1 Pooled AEX - terminal AEX capacity
1-F2 – in-process at AEX capacity limit
1-F3 – in process at pressure limit on Emphaze
2-F1 – 10SP02 @ 1.3 psid
2-F2 – 10SP02 @ 6 psid
2-F3 – 10SP02 @ 15 psid
2-F4 – 90ZB05 when 10SP02 @ 15 psid
3-F3 – 05SP01 when 90ZB08 @ 15 psid
3-F2 – 90ZB08 @ 15 psid
4-F1 – 60SP02 @ 3.5 psid
4-F2 – 60SP0 @ 7 psid



Conclusions

Cell shear is an important parameter to consider during clarification train design

- Product quality impact
- Downstream clarification stage stability
- Higher throughput does not always mean better clarification train

Strategies to minimize shear

- Use low pressure open grades for front stage, such as Zeta Plus 05SP01A, and size by cell breakthrough
- Limit front stage pressure to 5 psid and size by turbidity/ cell breakthrough
- Use tight charged grades at second stage to capture fine particles
- Conventional deep gradual funnel does not necessarily work

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