Moss Ultra-Sensitive SAPE Conjugates

Jim Lazar and Alvydas Ozinskas

Moss, Inc. • P.O. Box 189 • Pasadena, MD 21123

www.MossSubstrates.com

Part I: IL-6 Assay – Conjugate Survey

- IL-6 reagents sourced from Peprotech
- IL-6 Assay from Peprotech reagents with standards from 2000 to 2.7 pg/ml
- Washed assay
- 1 hour capture, 1 hour detection
- 30 min SAPE incubation
- Read on Luminex 100 with High PMT setting
- Two Thermo Conjugates and Ten Moss Conjugates were compared
- All Conjugates were tested at 2 ug/ml

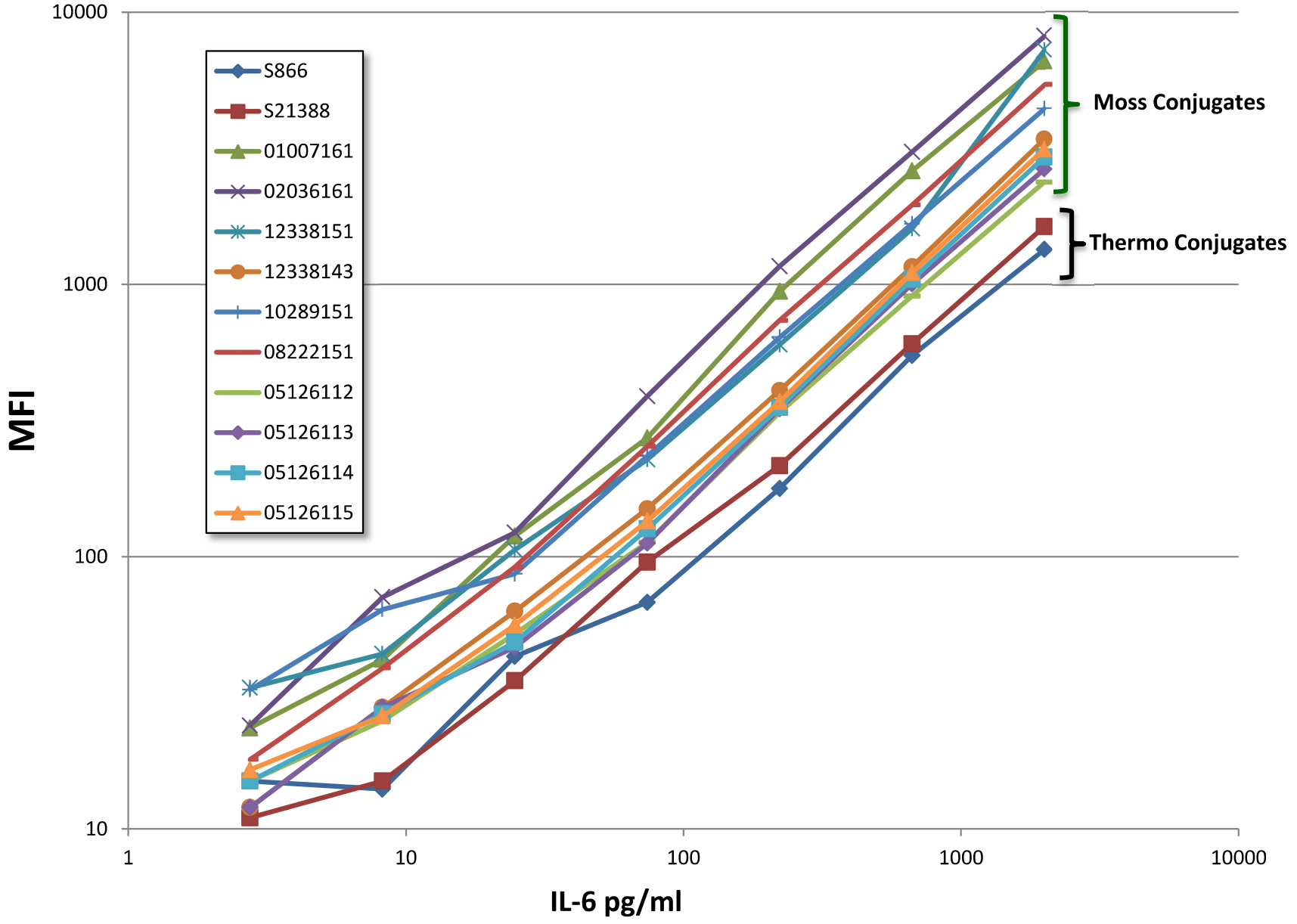
Day 1 –Conjugate Survey

Thermo Conjugates Tested

- S866 (Standard grade SAPE)
- S21388 (Premium grade SAPE)

Moss Conjugates Tested (Each variant can be reproducibly made)

Lot #	Description	Population
02036161	Moss 34 P	34 P
01007161	Moss 27 P	27 P
08222151	Moss 16 P	16 P
12338151	Moss 4 P	4 P
10289151	Moss 9.2 M	9.2 M
05126115	Moss 10.00 M	10.00 M
05126113	Moss 10.06 M	10.06 M
05126112	Moss 10.08 M	10.08 M
05126114	Moss 10.11 M	10.11 M
12338143	Moss 10.3 M	10.3 M


2 ug/ml SAPE – Comparison of All Conjugates
MFI
10000
1000
100
10
1
10
100
1000
10000
IL-6 pg/ml
S866
S21388
01007161
02036161
12338151
12338143
10289151
08222151
05126112
05126113
05126114
05126115
Moss Conjugates
Thermo Conjugates

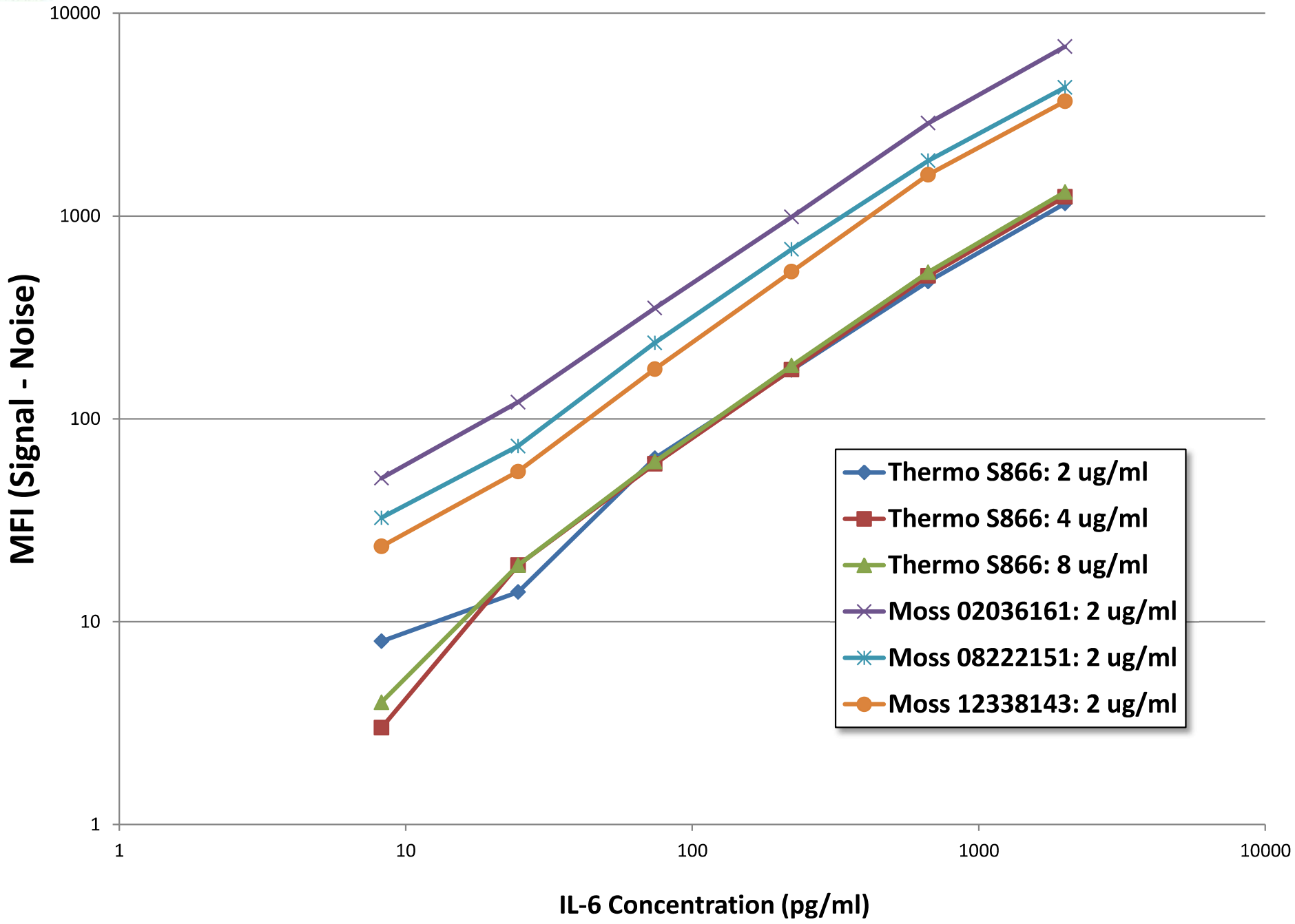
Part II – SAPE Titration with full IL-6 Assay

- IL-6 Assay from Peprotech reagents with standards from 2000 to 2.7 pg/ml
- Thermo Conjugates S866 and and Three Moss Conjugates were compared
- All Conjugates were tested at 2, 4 and 8 ug/ml.

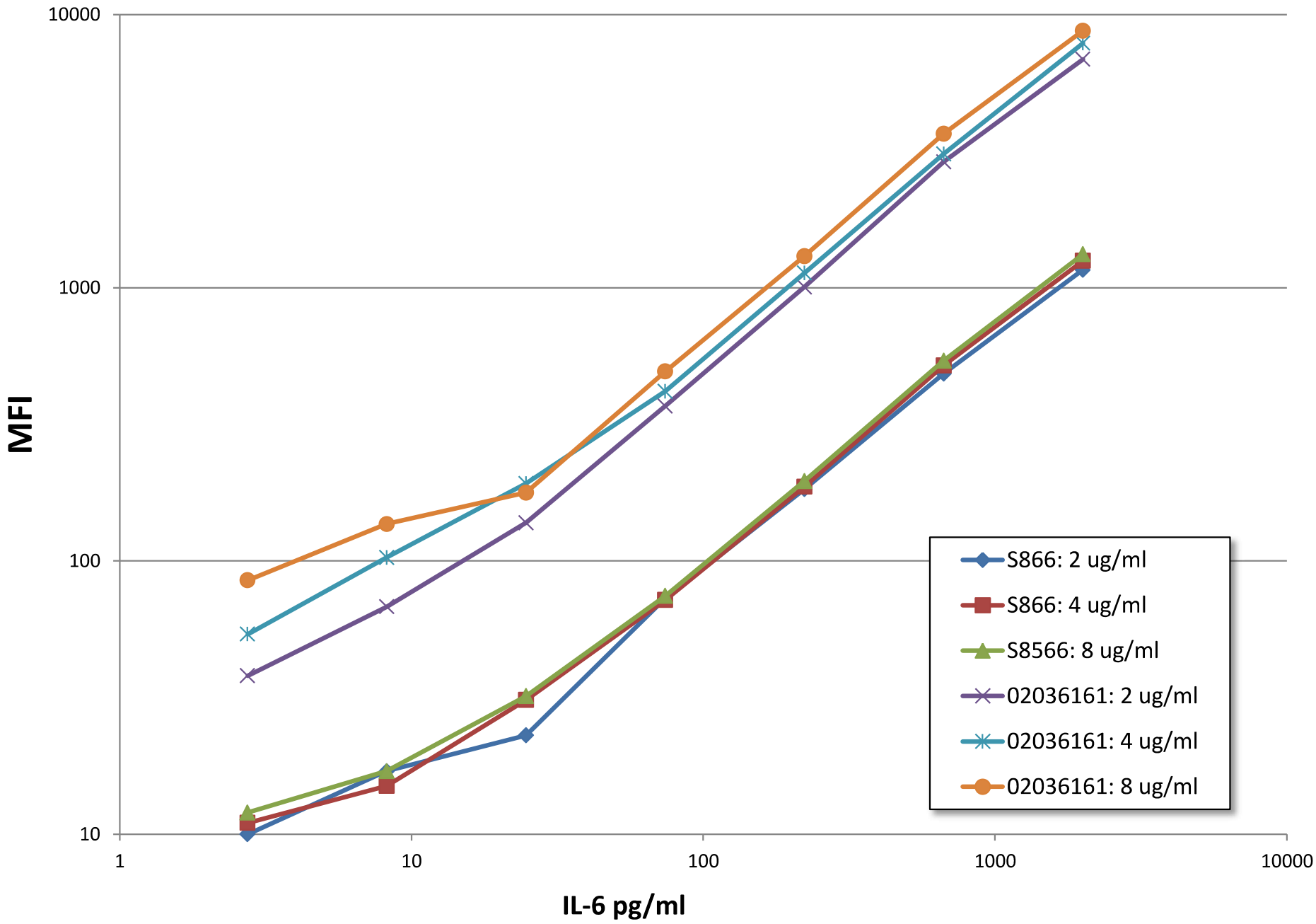
Conjugates Tested

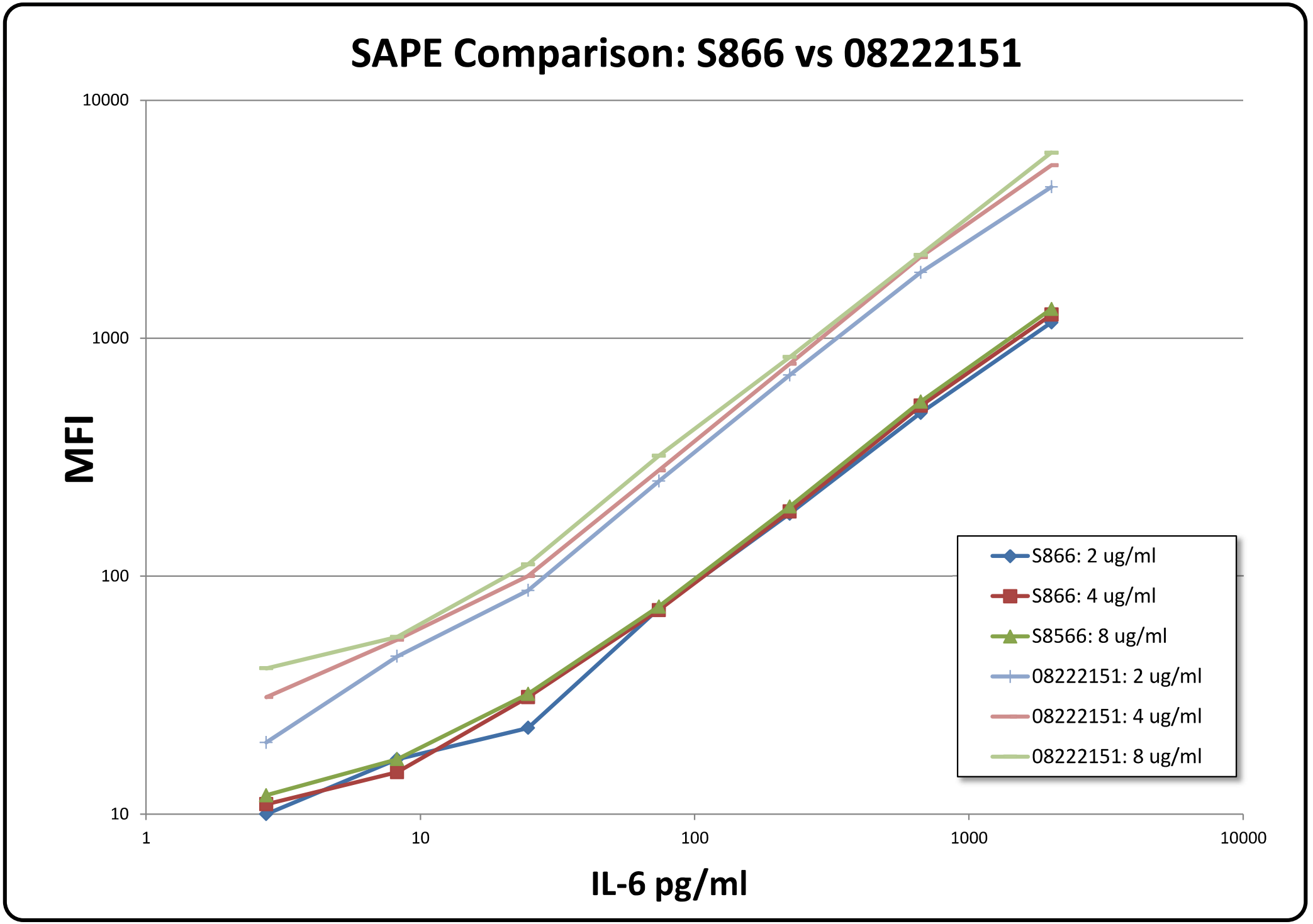
- Thermo
  - S866 (Standard grade SAPE) (11.3 M)
- Moss
  - SAPE-001, Lot # 02036161 (34P)
  - SAPE-001, Lot # 08222151 (16P)
  - SAPE-003, Lot # 12338143 (10.3 M)

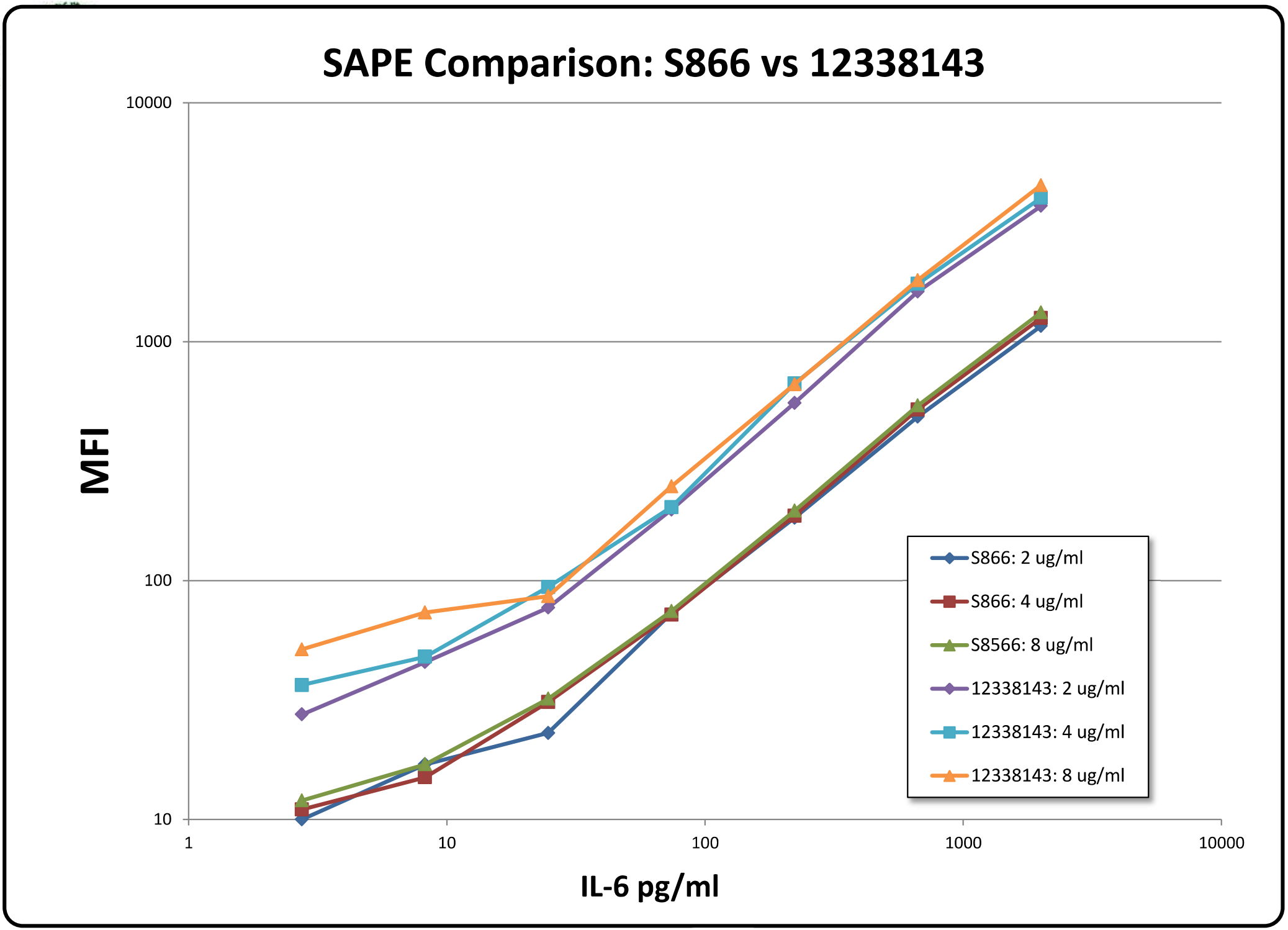
SAPE Comparision: Moss IL-6 Assay

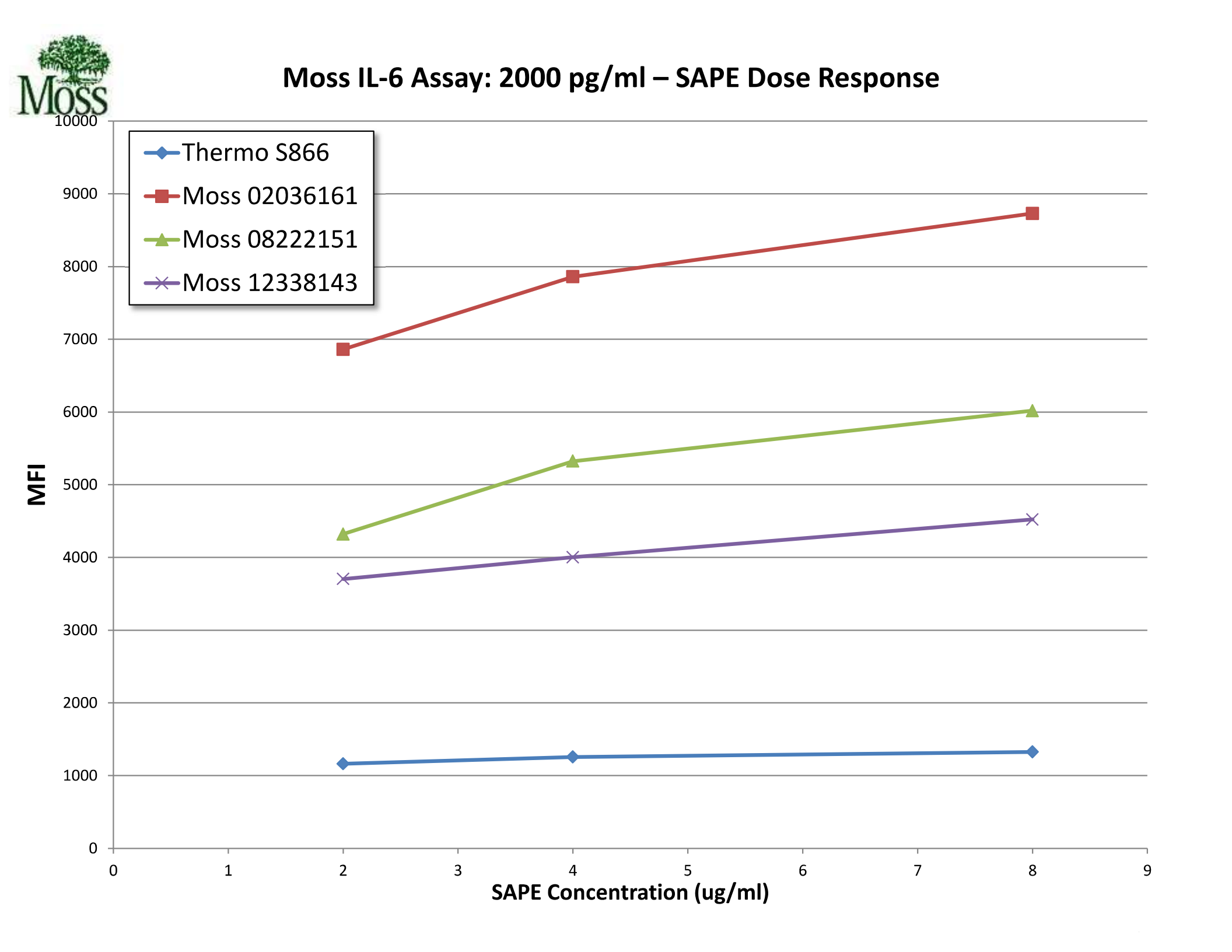


SAPE Comparison: S866 vs 02036161

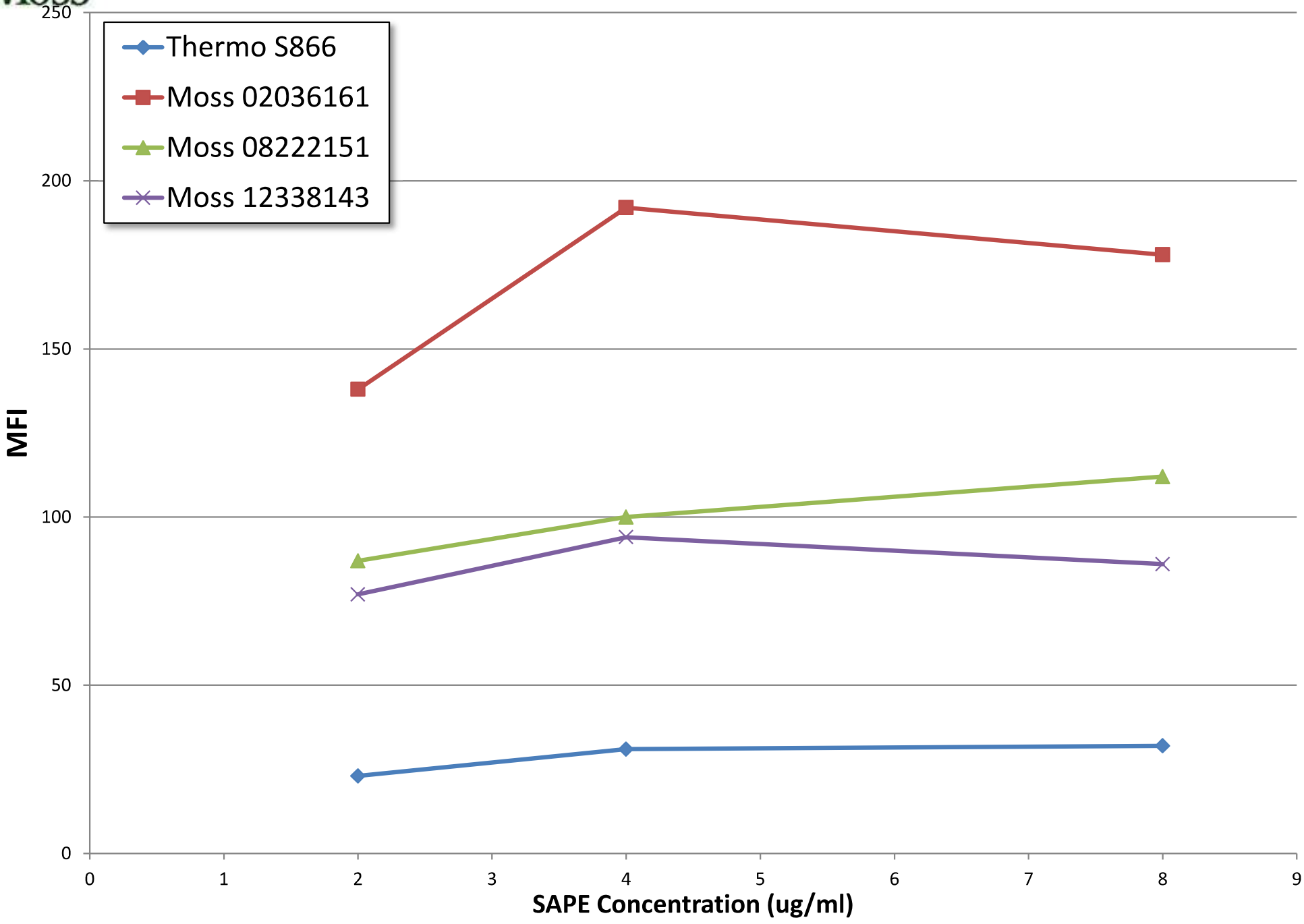



SAPE Comparison: S866 vs 08222151
10000
1000
100
10
MFI
1
10
100
1000
10000
IL-6 pg/ml
S866: 2 ug/ml
S866: 4 ug/ml
S8566: 8 ug/ml
08222151: 2 ug/ml
08222151: 4 ug/ml
08222151: 8 ug/ml


SAPE Comparison: S866 vs 12338143
MFI
10000
1000
100
10
1
10
100
1000
10000
IL-6 pg/ml
S866: 2 ug/ml
S866: 4 ug/ml
S8566: 8 ug/ml
12338143: 2 ug/ml
12338143: 4 ug/ml
12338143: 8 ug/ml

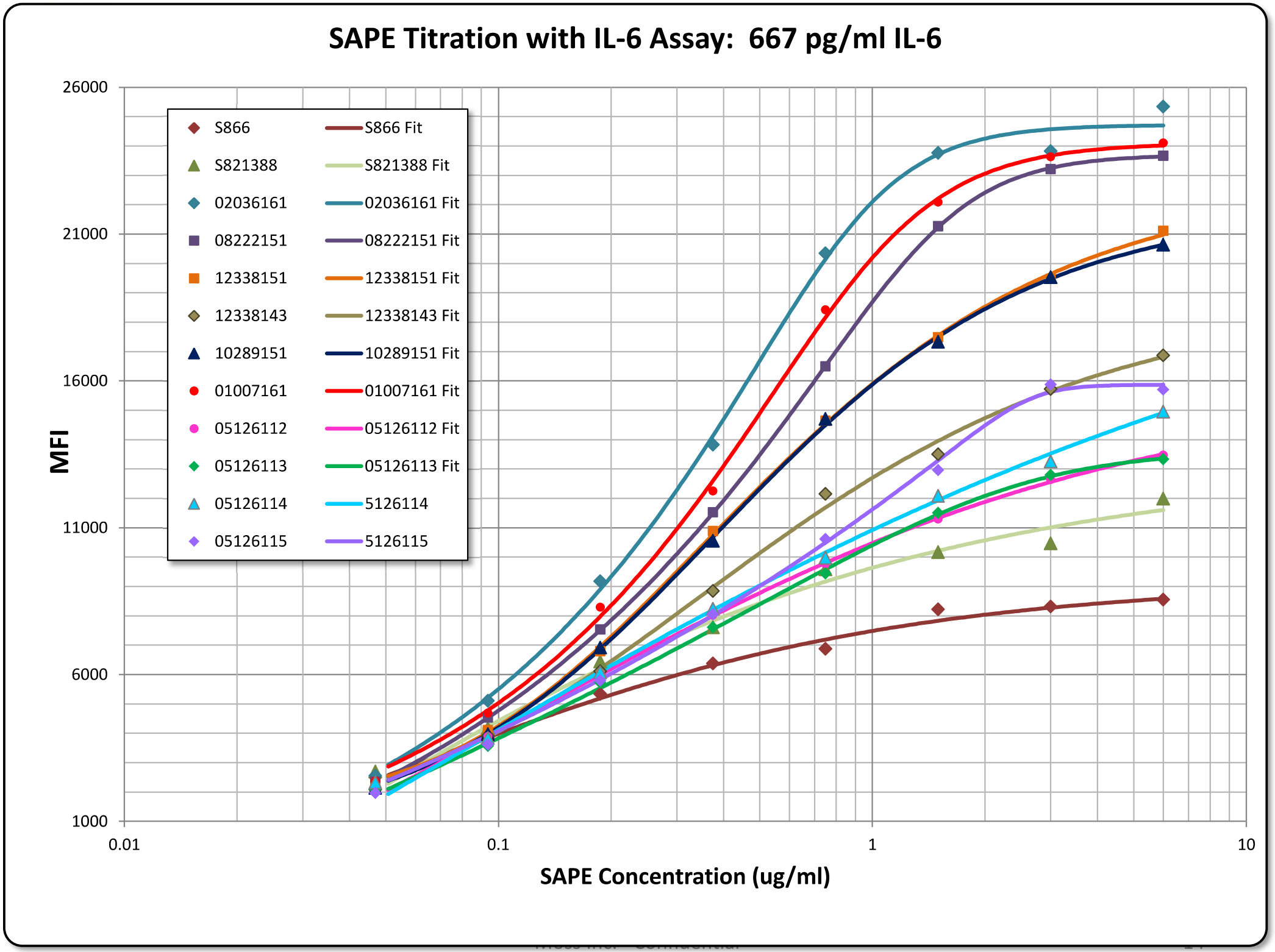

Moss IL-6 Assay: 2000 pg/ml – SAPE Dose Response
Thermo S866
Moss 02036161
Moss 08222151
Moss 12338143
MFI
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
0
1
2
3
4
5
6
7
8
9
SAPE Concentration (ug/ml)

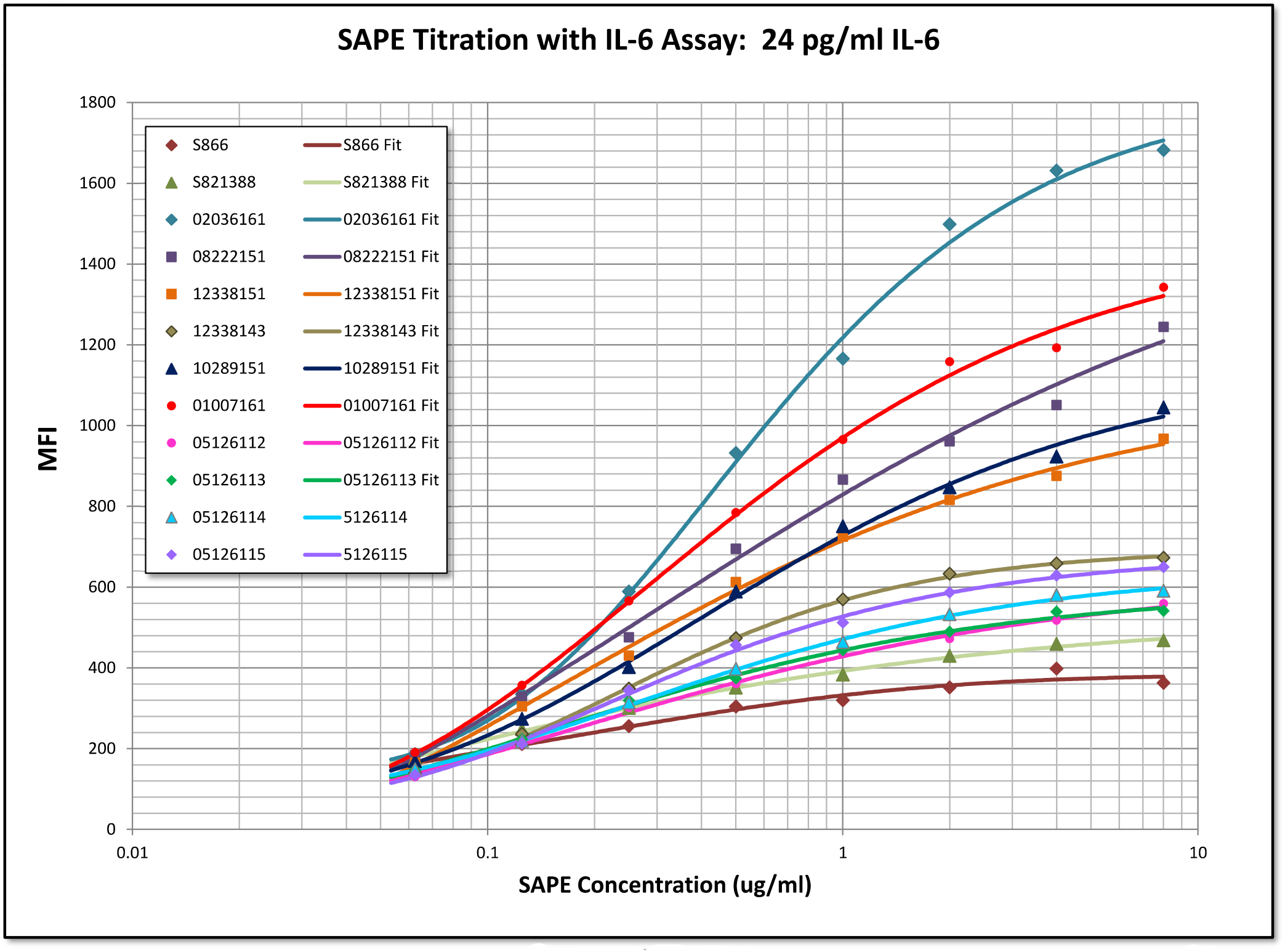
Moss IL-6 Assay: 25 pg/ml – SAPE Dose Response



Part III: SAPE Titration with IL-6 Standard 2

- IL-6 Assay from Peprotech reagents with standards from 2000 to 2.7 pg/ml
- Two Thermo Conjugates and Ten Moss Conjugates were compared
- Highest SAPE level was 6 ug/ml, serially diluted 3-fold.


SAPE Titration with IL-6 Assay: 667 pg/ml IL-6
MFI
26000
21000
16000
11000
6000
1000
0.01
0.1
1
10
SAPE Concentration (ug/ml)
S866
S866 Fit
S821388
S821388 Fit
02036161
02036161 Fit
08222151
08222151 Fit
12338151
12338151 Fit
12338143
12338143 Fit
10289151
10289151 Fit
01007161
01007161 Fit
05126112
05126112 Fit
05126113
05126113 Fit
05126114
5126114
05126115
5126115


SAPE Titration with IL-6 Assay: 24 pg/ml IL-6
MFI
SAPE Concentration (ug/ml)
1800
1600
1400
1200
1000
800
600
400
200
0
0.01
0.1
1
10
S866
S866 Fit
S821388
S821388 Fit
02036161
02036161 Fit
08222151
08222151 Fit
12338151
12338151 Fit
12338143
12338143 Fit
10289151
10289151 Fit
01007161
01007161 Fit
05126112
05126112 Fit
05126113
05126113 Fit
05126114
5126114
05126115
5126115

Conclusions

- In an IL-6 ELISA bead assay, the Moss Conjugates give 2-8 fold higher signals than either of the Thermo conjugates across a wide dynamic range.
- The premium Thermo conjugate gives slightly higher signal in an IL-6 assay than the standard Thermo conjugate, S866.
- At the normal concentrations used in assays (4 – 8 ug/ml), the signal is not sensitive to the conjugate concentration (the assay is saturated with SAPE).
- However, at lower concentrations the dose-response curve is relatively steep.
- Moss Conjugates give substantially higher signal than other commercially available conjugates.
- Moss Conjugates can be reproducibly prepared to generate specified levels of signal.

Contact Information

Sales and Distribution:

Vijay Hingorani

410-768-3442

Vijay4you@aol.com

www.MossSubstrates.com

Technical:

Jim Lazar, Ph.D.

410-768-3442

jlazar@mossbio.com