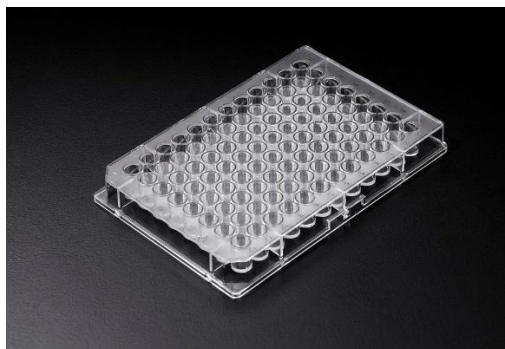


1. Product: Immunoplate

SPL Life Sciences Immunoplates are designed for enzyme-linked immunosorbent assay (ELISA) and other immunological applications requiring reliable protein binding performance. This bench test was conducted to evaluate the stability and binding performance of Uni Binding, Medi Binding, and Maxi Binding Immunoplates after accelerated aging and room-temperature storage. The evaluation was performed using an IgG, Ferritin based ELISA assay to verify product performance and support shelf-life determination.



2. Materials

1) Cell Culture ware

Type	Sample Selection Method
SPL Immunoplate	Random Sampling
A사	Random Sampling
B사	Random Sampling

2) Antibody

- IgG from murine serum: sigma I8765-5MG
- Anti-mouse IgG (Fab specific) peroxidase, antibody produced in goat: sigma A9917-1ML
- Ferritin standard stock solution (BIOCHECK, cat. 90013) 농도: 0, 5, 15, 30, 50, 75, 105ng/ml
- Ferritin zero standard/sample diluents (BIOCHECK, cat.MB-30013-1)
- Capture Ab: Rabbit Polyclonal Anti-Ferritin (BIOCHECK, cat. 70326), 1ug/ml
- Detection Ab: Mouse Monoclonal Anti-Human Ferritin (BIOCHECK, cat.70226) 0.5ug/ml-

3) Reagent

- Sodium carbonate buffer: sigma-C3041
- 1X PBS: Lonza 17-517Q (10x PBS)
- Tween20: m.biotech 43026
- BSA: sigma-A7030
- TMB reagent: KPL-50-76-00
- Coating buffer: sodium carbonate buffer (1 capsule in 100D.W.)
- Washing buffer: PBST (1XPBS+ 0.05% Tween 20)
- Blocking buffer: BSA (bovine serum albumin) + PBST
- TMB reagent: A+ B=1:1
- Stop solution: 2N H₂SO₄

4) Instrument

- ELISA reader: Sunrise RC

2.2. Protocol

- (1) **Coating Step:** Dilute the antibody in coating buffer to concentrations of 0, 0.0625, 0.125, 0.25, 0.5, and 1µg/mL. Dispense 100µL into each well, seal the plate, and incubate at room temperature (RT) for 2 hours.
- (2) **Washing Step:** Wash the wells three times with washing buffer.
- (3) **Blocking Step:** Add 100µL of blocking buffer to each well, seal the plate, and incubate at RT for 1 hour.
- (4) **Washing Step:** Wash the wells three times with washing buffer.
- (5) **Secondary Antibody Reaction:** Dilute the HRP-conjugated anti-mouse IgG antibody 1:3000 in 1× PBS. Add 100µL to each well, seal the plate, and incubate at RT for 1 hour.

(6) **Washing Step:** Wash the wells three times with washing buffer.

(7) **Substrate Reaction:** Prepare the TMB substrate by mixing Reagent A and Reagent B at a 1:1 ratio. Add 100 μ L to each well, seal the plate, and incubate at RT for 10 minutes.

(8) **Stopping Reaction:** Add 100 μ L of stop solution (2 N H₂ SO₄) to each well.

(9) **Measurement:** Measure the absorbance at 450 nm using an ELISA microplate reader.

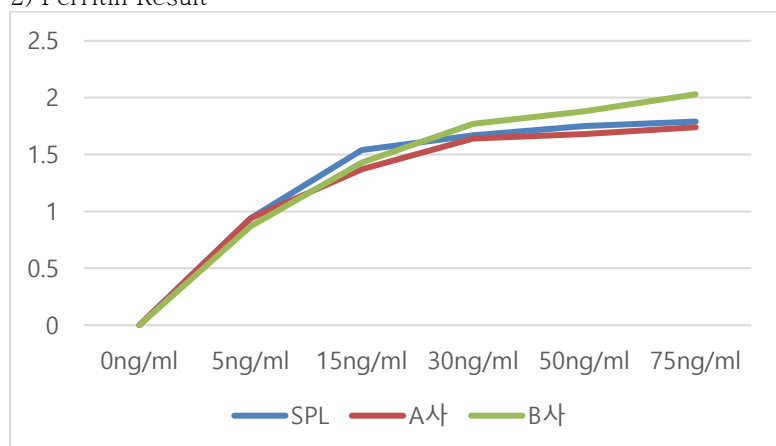
2.3. Results

1) IgG Result



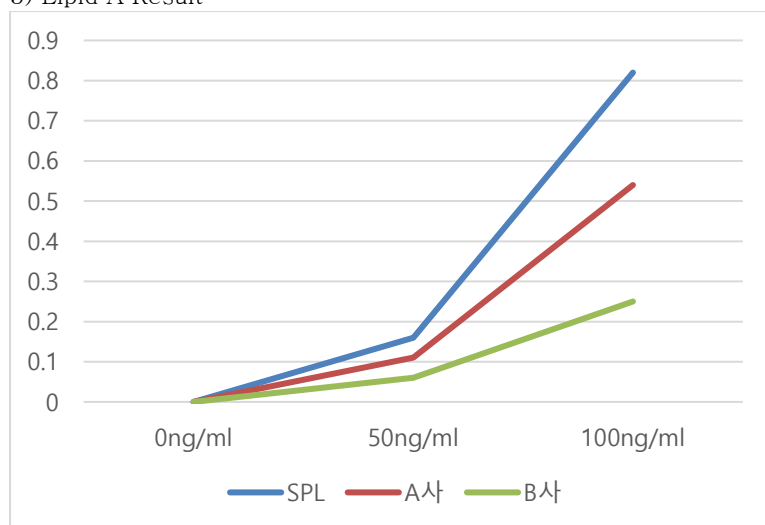
AVE	0ug/ml	0.0625ug/ml	0.125ug/ml	0.25ug/ml	0.5ug/ml	1ug/ml
SPL	0.072	1.743	2.283	2.618	2.795	3.005
A사	0.075	1.766	2.277	2.650	2.839	2.948
B사	0.07	1.765	2.266	2.633	2.811	2.977

2) Ferritin Result



AVE	0ng/ml	5ng/ml	15ng/ml	30ng/ml	50ng/ml	75ng/ml
SPL	0	0.94	1.54	1.67	1.75	1.79
A사	0	0.94	1.37	1.64	1.68	1.74
B사	0	0.87	1.43	1.77	1.88	2.03

3) Lipid A Result



AVE	0ng/ml	50ng/ml	100ng/ml
SPL	0.00	0.16	0.82
A사	0.00	0.11	0.54
B사	0.00	0.06	0.25

- SPL Immunoplate generated OD values comparable to those obtained using the A, B Immunoplate.
- No significant difference in binding performance was observed throughout the tested IgG, Ferritin, Lipid A concentration range.

4) Comparison of CV Values (%)

CV (%)	0.0625ug/ml	0.125ug/ml	0.25ug/ml	0.5ug/ml	1ug/ml
SPL	3.81	2.35	1.55	1.38	1.74
A사	3.54	2.78	1.95	1.07	2.16
B사	3.5	2.7	1.85	1.2	1.8

– The CV values were evaluated across protein concentrations ranging from 0.0625 to 1 µg/mL. SPL Immunoplate showed CV values ranging from 1.38% to 3.81%, while A and B showed ranges of 1.07–3.54% and 1.20–3.50%, respectively.

– Across the tested concentration range, **SPL demonstrated a CV profile comparable to those of the A and B reference products.**

– The CV values remained within a relatively narrow range and showed a similar concentration-dependent pattern, indicating **comparable assay precision and reproducibility among the evaluated products.**

4. Conclusion

– SPL Black and SPL Clear Immunoplates demonstrated ELISA performance comparable to that of the competitor products evaluated in this study.

– Overall, the results confirm that SPL Immunoplates provide ELISA performance comparable to that of leading global competitor products, with equivalent protein-binding capacity and analytical performance under the tested conditions.

www.spllifesciences.com

For technical assistance, contact SPL R&D Center at:

Tel: +82-31-533-4800; Fax: +82-31-533-1430; e-mail: spl@ispl.co.kr

To place an order, contact your local distributor or

Tel: +82-31-533-4800; Fax: +82-31-533-1430; e-mail: business@ispl.co.kr