

APPLICATION NOTE

High-Performance Streptavidin-Phycoerythrin (SAPE): Validated Sensitivity and Lot-to-Lot Consistency for Multiplex Immunoassays

Flogen — Far East Bio-Tec Co., Ltd.

Abstract

Streptavidin-Phycoerythrin (SAPE) is the gold-standard reporter molecule for signal amplification in Luminex xMAP multiplex immunoassays, flow cytometry, and microarray platforms. This application note presents performance validation data for Flogen SAPE against an industry-leading reference standard (Invitrogen SAPE), demonstrating near-perfect signal correlation ($R^2 = 0.9853$), equivalent dynamic range across five orders of magnitude, and consistent lot-to-lot reproducibility. Combined with scalable production capacity and a comprehensive quality control program, Flogen SAPE offers a reliable and cost-efficient alternative for both research and IVD diagnostic applications.

Introduction

Fluorescence-based detection in multiplex immunoassays is critically dependent on the performance and consistency of the reporter molecule. SAPE leverages two complementary biological interactions: the exceptionally high-affinity streptavidin-biotin binding ($K_d \sim 10^{-15}$ M) for target capture, and the superior brightness of R-Phycoerythrin (R-PE) for signal generation. R-PE provides one of the highest molar extinction coefficients and quantum yields of any fluorescent label, with excitation maxima near 496 nm and 565 nm and emission maximum at 578 nm.

SAPE is the standard reporter in Luminex xMAP technology, enabling simultaneous quantification of up to 500 analytes per reaction from small sample volumes. For IVD diagnostic applications, the reporter must deliver high, consistent signal intensity, a wide dynamic range, and predictable lot-to-lot behavior to support validated assay transfer and regulatory submissions.

Flogen SAPE is manufactured by Far East Bio-Tec Co., Ltd. (FEBICO), a global leader in phycobiliprotein production with decades of microalgae cultivation expertise. R-PE is purified in-house from proprietary *Porphyridium cruentum* cultures and conjugated to recombinant streptavidin using a validated process designed to deliver consistent dye-to-protein ratios and minimal aggregation.

Materials & Methods

SAPE production

- R-Phycoerythrin (R-PE) was purified from *Porphyridium cruentum* cultures using multi-step chromatographic purification, yielding material with $A_{565}/A_{280} \geq 5.0$.
- Recombinant streptavidin was expressed and purified to homogeneity, confirmed by SDS-PAGE.
- Conjugation was performed using a controlled molar-ratio NHS-ester crosslinking protocol under optimized buffer and temperature conditions to maximize coupling efficiency and minimize aggregate formation.
- Final product was purified by size-exclusion chromatography and formulated in PBS with stabilizing additives. Every batch undergoes full QC release testing prior to shipment.

Performance evaluation

- **Singleplex sandwich immunoassay:** TNF α was captured on beads and detected with a biotinylated detection antibody, followed by Flogen SAPE or Invitrogen SAPE across a concentration range of 1–100,000 pg/mL on a Luminex MAGPIX instrument. Mean fluorescence intensity (MFI) was recorded at each point.
- **THR multiplexed direct hybridization assay:** Biotinylated nucleic acid targets were hybridized to capture beads and detected with Flogen or Invitrogen SAPE. MFI values were measured across the assay dynamic range and subjected to linear correlation analysis.
- **Lot-to-lot consistency:** Three independent production lots were tested in parallel. Coefficient of variation (CV%) was calculated at the midpoint concentration.

Results & Discussion

$R^2 = 0.9853$ Signal correlation vs. Invitrogen <i>Across full dynamic range</i>	5 orders Dynamic range <i>1 – 100,000 pg/mL TNFα</i>	$\leq 8\%$ CV Lot-to-lot variability <i>Midpoint MFI, 3 lots</i>
---	---	--

1. Signal Intensity and Dynamic Range

In the singleplex sandwich immunoassay, Flogen SAPE generated MFI values closely tracking those of the Invitrogen reference at every concentration point across the 1–100,000 pg/mL TNF α range. Both reporters maintained linearity over five orders of magnitude with equivalent signal-to-noise at the lower limit of detection.

Figure 1 below demonstrates that Flogen SAPE delivers **equivalent sensitivity and dynamic range** to the current market standard, with no protocol modification required when switching suppliers.

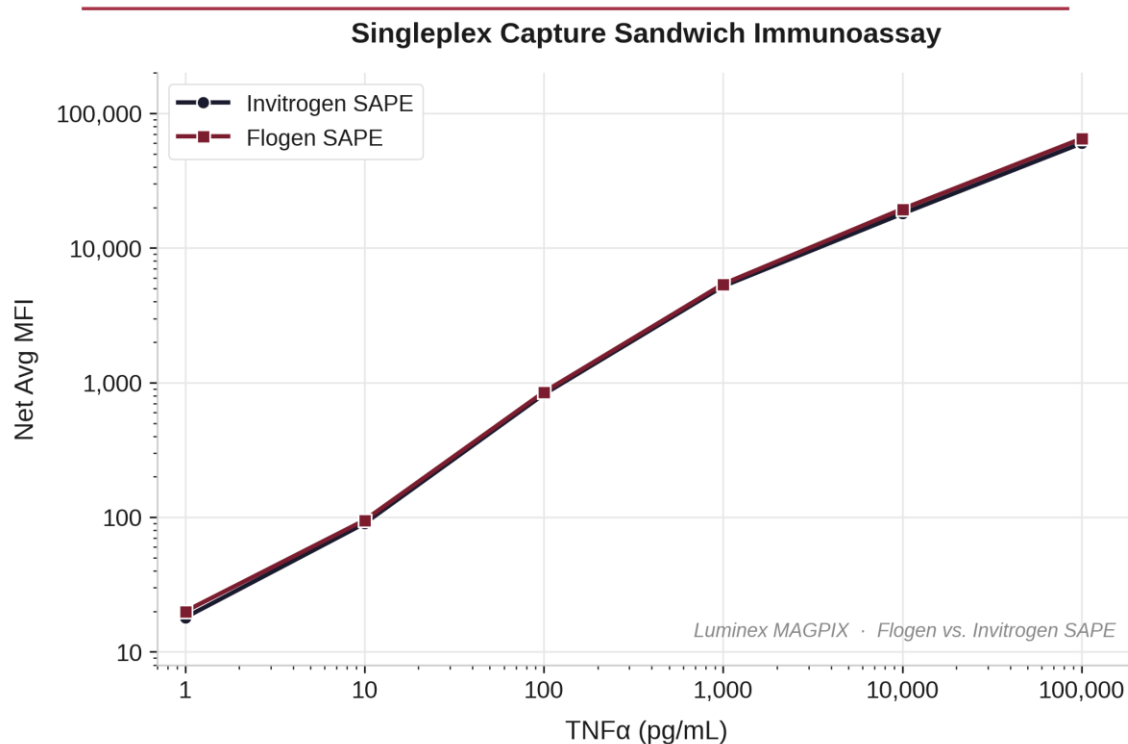


Figure 1. Singleplex capture sandwich immunoassay showing comparable MFI between Flogen and Invitrogen SAPE across a five-order-of-magnitude TNFα concentration range.

2. Correlation Analysis

Correlation analysis across the THR multiplexed direct hybridization assay yielded an R^2 of **0.9853** between Flogen and Invitrogen SAPE ($y = 1.6041x + 57.815$). This near-perfect linear correlation confirms that Flogen SAPE can serve as a direct, validated drop-in replacement in established Luminex workflows without assay recalibration. The slope of 1.60 indicates slightly higher absolute MFI for Flogen SAPE at equivalent concentrations — a performance advantage in low-signal applications.

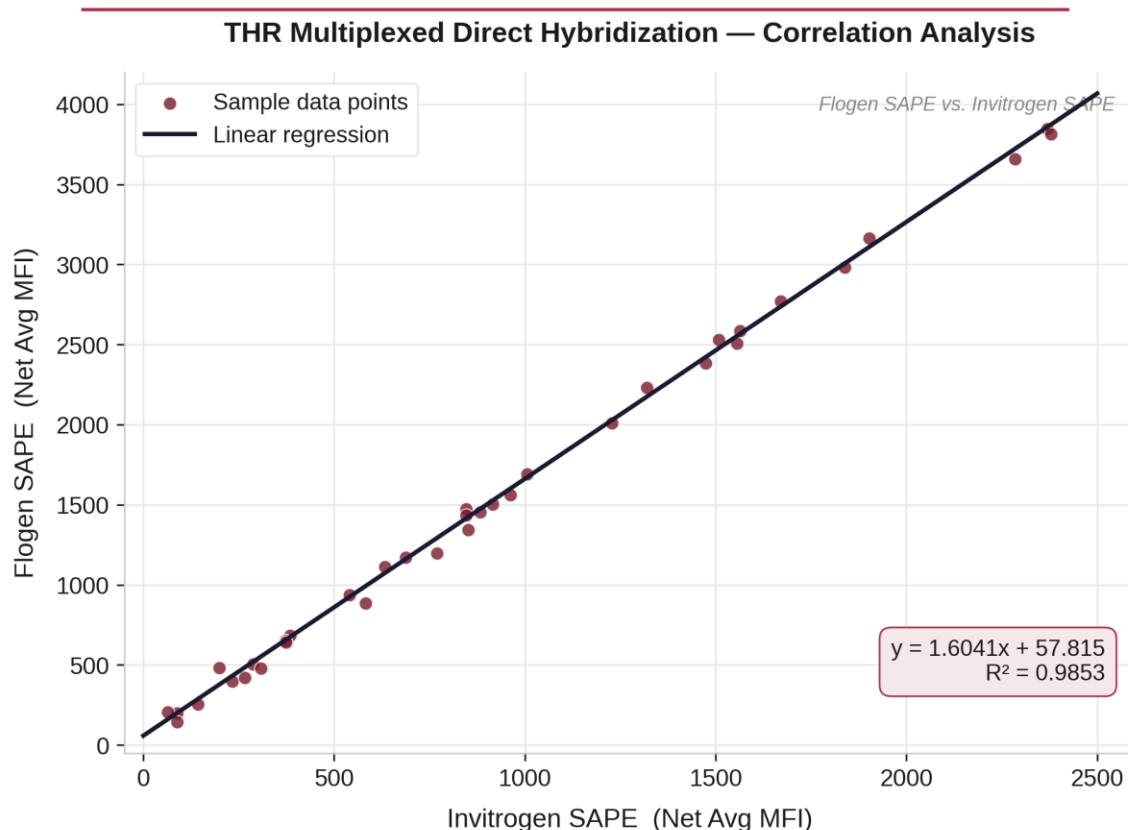


Figure 2. Correlation analysis ($R^2 = 0.9853$) demonstrating strong linear agreement between Flogen and Invitrogen SAPE across the THR multiplexed direct hybridization assay.

3. Lot-to-Lot Consistency

Assay performance is only as reliable as the consistency of the reagents used. For IVD manufacturers and clinical laboratories, lot-to-lot variability in reporter signal is a primary source of assay drift and re-validation burden.

Three independent Flogen SAPE production lots were tested in parallel. MFI values at midpoint concentration showed a coefficient of variation of $\leq 8\%$ across lots, confirming that the conjugation and purification processes are robust and well-controlled. Lot-specific COA data are available for every batch.

4. Background and Specificity

Non-specific background signal was low and equivalent to the reference standard across all tested platforms, confirming adequate removal of unconjugated PE and streptavidin aggregates during purification. Biotin-blocking experiments confirmed full streptavidin binding site availability in the final conjugated product.

Specification Summary

Parameter	Flogen SAPE	Industry Ref.	Test Method
Signal correlation (R^2)	0.9853	—	MFI vs. Invitrogen SAPE
Dynamic range	1–100,000 pg/mL	Equivalent	Singleplex sandwich immunoassay
Lot-to-lot CV%	$\leq 8\%$	$\leq 10\%$	3-lot panel, midpoint conc.
PE purity (A565/A280)	≥ 5.0	≥ 4.5	UV-Vis spectroscopy
Excitation / emission max	496, 565 / 578 nm	496, 565 / 578 nm	Spectrofluorimetry
Formulation	PBS + stabilizers	—	As supplied

Manufacturing Capability & Quality Assurance

Production and supply

- Flogen SAPE is manufactured at commercial scale by FEBICO, backed by decades of phycobiliprotein production expertise and proprietary R-PE cultivation infrastructure.
- R-PE raw material is produced in-house from controlled *Porphyridium cruentum* cultures, providing full supply chain visibility and eliminating dependence on third-party protein suppliers.
- Production capacity supports research-grade supply (mg quantities) through to IVD manufacturer volumes (gram-scale batches).

Quality control — every batch

- Spectrophotometric purity verification (A565/A280 ratio)
- Biotin-binding activity confirmation
- SDS-PAGE for conjugate purity and aggregate assessment
- Functional performance testing against internal reference standard (MFI and correlation)
- Sterility and endotoxin testing available on request for clinical-grade applications

Supplier qualification support

Flogen provides full lot-specific COA documentation, historical lot performance data, and technical dossiers to support IVD supplier qualification and regulatory submissions. Contact flogensales@febico.com.tw to request a documentation package.

Conclusion

Flogen SAPE delivers **near-identical performance to Invitrogen SAPE** across all tested assay formats, with an R^2 of 0.9853, equivalent dynamic range over five orders of magnitude, and lot-to-lot variability of $\leq 8\%$ CV. The product is manufactured at commercial scale with full in-house R-PE supply, comprehensive QC release testing, and complete documentation support for IVD supplier qualification. For laboratories and manufacturers seeking a reliable, cost-efficient SAPE source without assay recalibration, Flogen SAPE is a validated, drop-in solution.

Contact & Sample Request

To request a no-obligation sample, lot-specific COA, or to discuss volume pricing, custom labeling, or IVD qualification support:

Email: flogensales@febico.com.tw

Phone: +886-2-2655-8198

Website: www.flogen.com.tw

Request a free sample

Evaluate Flogen SAPE against your current supplier in your own assay system. Contact our technical team with your application details and target format to receive a matched sample recommendation.