RESOLIAN
BIOANALYTICS

ELISpot-Antigen Specific Response Assays

A powerful tool for unveiling cellular immune activity

The ELISpot assay stands out as a highly sensitive technique for analyzing immune function. It offers single-cell resolution by directly counting individual cells that secrete cytokines in response to specific stimuli, including large molecules and new modalities of the pharmaceutical agents from recombinant proteins to cell and gene therapies.

This unique capability unlocks a range of valuable applications: infectious vaccines, cancer vaccines, immunogenicity assessments, and predicting clinical benefit after therapeutic immune modulation.

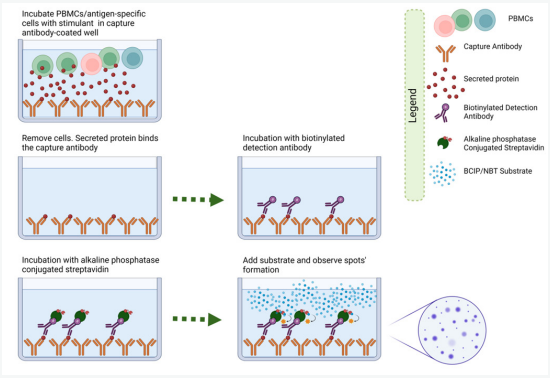

Figure 1: Overview of enzyme-linked immunospot (ELISpot) assay.

ELISpot - A Multifaceted Approach to Immunological Evaluation

ELISpot Applications:

Efficacy Evaluation (Vaccine/Immunomodulators)	ELISpot precisely measures antigen-specific T cell responses, enabling evaluation of vaccine efficacy and immunomodulator potency, ultimately leading to better immunotherapies.
Safety/Immunogenicity Assessment (Cell and Gene Therapies)	ELISpot predicts immunogenicity risks in cell and gene therapies by enabling detection of T cell responses against introduced cells or gene products. This ensures patient safety during clinical trials.
Transplant Rejection Monitoring (Pre/Post Transplantation)	ELISpot monitors transplant rejection risk by quantifying recipient T cell response to the donor organ, enabling early detection and improved transplant outcomes.
Translational Research (Clinical implications)	ELISpot underpins immunological research. It facilitates the investigation of fundamental immunological processes, such as T cell activation and antigen recognition, and their clinical implications, bridging the gap between basic science discoveries and the development of novel immunotherapies.

Unveiling Low-Frequency Immune Responses:
Reliable Sample Processing Makes the Difference

- Studying subtle immune reactions can be tricky. Traditional methods often struggle due to damage to live blood cells or frozen PBMCs during shipping and freezing. At Resolian, we have achieved proficiency in sample preparation for reliable immune response analysis.
- Traditional PBMC recovery methods often suffer from high variability and low cell viability. Our comprehensive processing and defrosting protocol prioritizes maintaining high cell viability and excellent recovery. This ensures the critical functionality of antigen-specific cells is preserved, leading to:
  - High-quality, consistent data: Minimize variability and ensure reliable results.
  - Enhanced sensitivity: Confidently detect even weak immune responses.

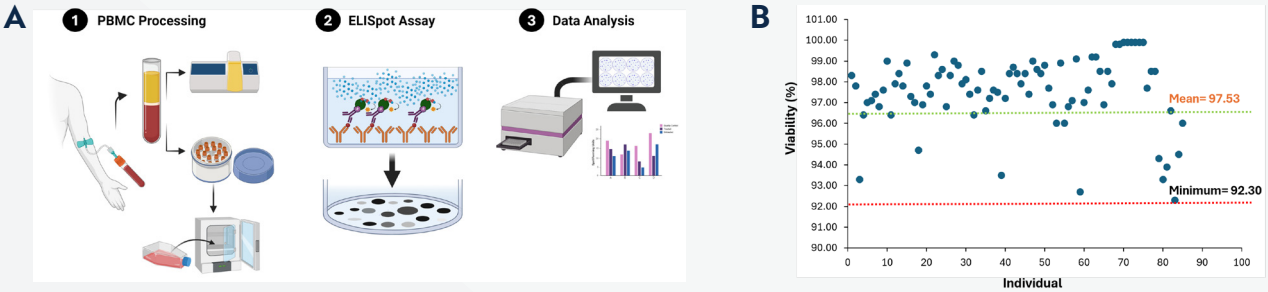

Figure 2:
A Our-In-House, Three-Step ELISpot Assay.
(1) PBMC recovery and viability determination. (2) ELISpot assay performance. (3) Data acquisition, analysis and review.
B Viability assessments. This graph shows the viability of PBMCs isolated from 85 different individuals following an overnight recovery process. The green line represents the average percentage of viable PBMCs across all 85 individuals. The red line represents the lowest percentage of viable PBMCs observed in any of the 85 samples.

Speed and Expertise to Power Your Research

Dedicated sample management
We handle hundreds of samples weekly to meet your study demands.

Data integrity compliance
We prioritize data security with robust CFR 21 Part 11 compliance.

Complete regulatory compliance
Our studies are conducted in accordance with GLP and GCP regulations, ensuring smooth progress from pre-clinical stages to final product release and clinical trials in accordance with the FDA, EMA, and MHRA recommendations and guidelines.

Why Resolian? Rigorous Method development and Validation

- **Enhanced ELISpot Sensitivity Discerns Inter-Donor Variability**
  Our optimized method detects subtle variations in immune responses between donors despite background interference. This is achieved through a combination of optimized reagents, protocols, and advanced data analysis. This enhanced sensitivity allows for precise differentiation of individual immune profiles.
- **Data-Driven Validation**
  We establish validation criteria based on statistically significant reactivity levels, ensuring the robustness of your results. (Figure 3: A-D).
- **Inter and Intra-Assay Precision**
  Our optimized method provides solution to minimize variability and provides consistent performance with low CV percentage (Figure 3: A&B).
- **Evaluation of Samples with High Background of IFN-γ Secretion**
  Samples exhibiting elevated levels of background IFN-γ secreting cells will undergo confirmatory analysis using a pre-determined cut-off value (CCP). This CCP will be employed to definitively categorize samples where both peptide-specific responses and mock responses fall at or above the Limit of Detection (LOD) (Figure 3: C&D).
- **Serum-Free Culture - Minimizing Bias and Enhancing T Cell Function**
  Our optimized culture media tackles a problem of serum-derived bias by utilizing a serum-free media supplemented with a xeno-free alternative. This approach minimizes non-specific mitogenic or suppressive factors and background reactivity, ensuring high-fidelity assay performance (Figure 3: E-G).
- **Batch Testing**
  All reagents and kits are batch tested for unform performance (Figure 3: H).

Figure 3: Examples of parameters optimized in our IFN-γ ELISpot method to monitor AAV5-antigen T cell immunity represents the assay robustness.

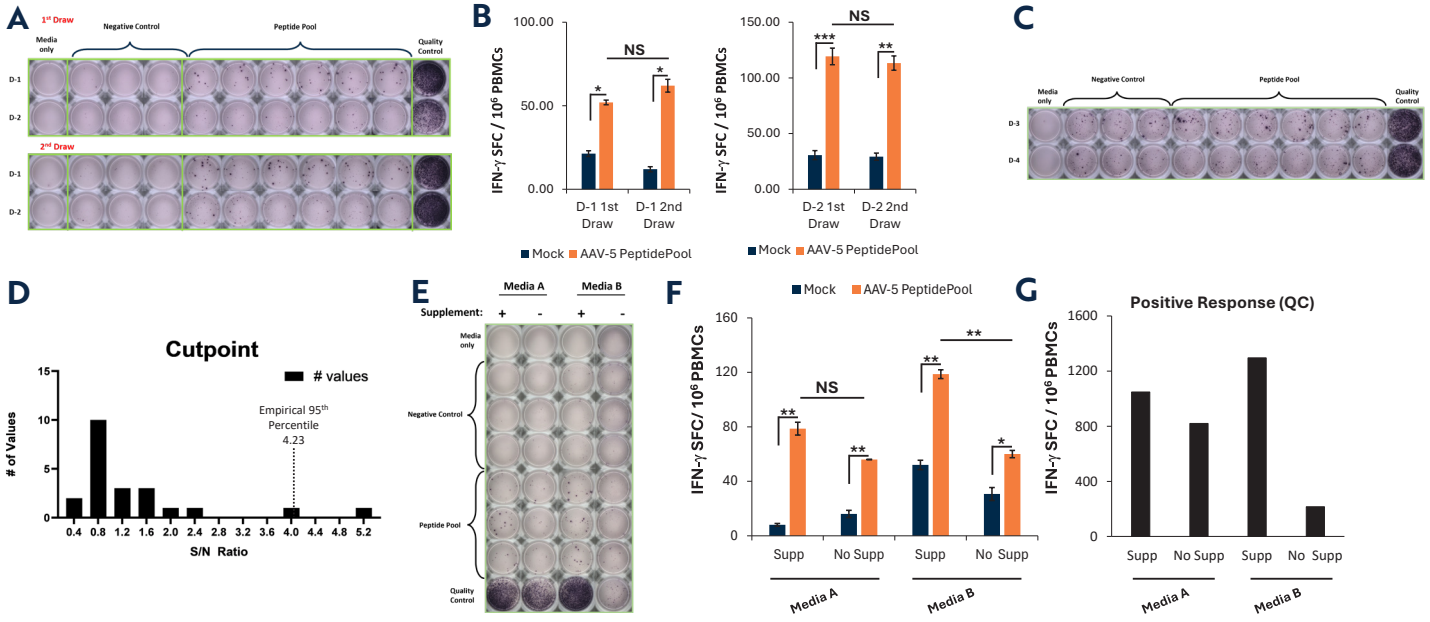

Figure 3:
(**A**) and (**B**) Our optimized method allows us to statistically identify varying levels of reactivity, while minimizing background interference. Conducting IFN-γ ELISpot for monitoring T Cell reactivity against Adeno-Associated Virus-5 (AAV5) in PBMCs isolated from two separate donors at two different time points. The results showcased notably precise [D-1 CV(1st Draw)=8.89%, CV(2nd Draw)= 16.03%; D-2 CV(1st Draw)=25%, CV(2nd Draw)= 22.05%] and consistent performance (D-1, %RE= 13.79; D-2, %RE= 5.15), achieving inter and intra-assay coefficient of variation (CV%) below 25%.
(**C**) and (**D**) CCP applied to samples with high background of IFN-γ-secreting cells. CCP adjudicates between peptide-specific and mock responses ≥ LOD. A sample is considered positive when the Antigen/Mock ratio is greater than or equal to the established cut-off point (CCP) of 4.23.
(**E**) And (**F**) Our method achieves superior T cell functionality and consistent assay results by utilizing a meticulously optimized serum-free culture media with targeted supplement.
(**G**) Anti-CD3 (mAb) stimulation served as a quality control to assess PBMC viability and T Cells response.
(**H**) Our method exposes non-specific signals from impure batches (peptide pool <70% purity).

Our team of experts can handle all aspects of your ELISpot assay.
From data acquisition and analysis to interpretation,
we'll ensure you get the most out of your data.

www.resolian.com

Learn more

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