

SINGULEX ERENNA[®] IMMUNOASSAY SYSTEM

Previously undetectable, quantified. One molecule at a time.

Complete solution for biomarker research:

- Instrument
- Assay Kits and Reagents
- Custom Services



The life science business of Merck KGaA,
Darmstadt, Germany operates as
MilliporeSigma in the U.S. and Canada.

**MILLIPORE
SIGMA**

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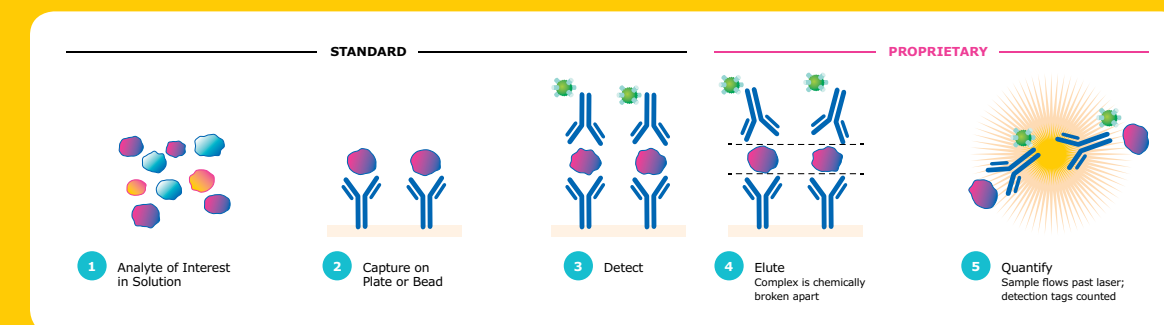
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Erenna® Immunoassay System Powered by SMC™ Technology

Single Molecule Counting (SMC™) Technology

Reduced background + increased signal

SMC™ technology provides maximum immunoassay performance while following a workflow very similar to traditional ELISA technology, as shown below. By combining a unique assay elution step and robust digital counting, SMC™ technology achieves improved signal-to-noise ratios over traditional immunoassay technologies, thus providing quantification at both low and high levels of expression on one complete system.



SMC™ Assay Workflow. During the capture and detection steps, specific antibodies translate each biomarker into a signal. During the modified elution step, fluorescent dye-labeled detection antibodies are released from the immune complexes. The eluate is then drawn into the Erenna® System capillary tube, which contains a very small interrogation space that is illuminated by a laser. Single fluorescently labeled molecules are detected as they generate intense flashes of light when passing through the interrogation space. Detected signals with peak intensity above the threshold of background fluorescence are counted as digital events.

Digital counting improves sensitivity and dynamic range

The Erenna® instrument captures the sum of all digital events counted. At high concentrations, a proprietary algorithm computes the total sum of all photons recorded. Thus, SMC™ technology improves assay sensitivity and extends dynamic measuring range far beyond what could be achieved with traditional technologies.

Erenna® Immunoassay System Specifications

Minimum Instrument Performance Specifications

| Metric | Specification |
|--------------------------|----------------------|
| Slope | > 20 DE/fM* |
| Background | < 100 DE |
| Limit of detection (LoD) | < 1 fM* |
| Precision | < 7% CV [†] |
| Dynamic range | > 4 logs |

*Determined from calibrator set: 0-300 fM of 150 kD antibody labeled with fluorophore

[†]DE measurements from 30 fM calibrator, n=20

Network/PC Requirement

- Microsoft Windows® 7 Operating Systems
- A static IP address and an FTP server
- Sgx link Operating and Analysis software included

Read Plate Format

- 384-well plate

Assay Format

- Plate-based assays
- Bead-based assays

Instrument Dimensions and Weight

- H: 400 mm (15.75 in.)
- W: 540 mm (21.25 in.)
- D: 575.6 mm (22.7 in.)
- W: 31.3 kg (69 lb.)

Power Requirement

- U.S.: 115 VAC, 50-60 Hz (operating range 90-125 V)
- Int.: 230 VAC, 50-60 Hz (operating range 180-250 V)

Validation Criteria for Verified Immunoassays

A comprehensive set of criteria evaluating ultimate quantitative performance is used to qualify Verified Immunoassays.

Lower limit of quantitation

Lowest point on standard curve with CV <20% and accuracy within 20% of expected values.

Inter- and intra-assay precision

Samples run on multiple plates over multiple days. Spiked and un-spiked samples within 20% across experiments.

Spike recovery

Minimum of 10 samples spiked with acceptable recovery between 80-120%.

Dilutional linearity

Assays target a quantifiable range of 3-4 logs.

Endogenous range

Minimum of 10 samples from individual donors assessed for ability to quantify baseline biomarker levels.

SMC™

Immunoassay Menu

For the Singulex Erenna® Immunoassay System

MilliporeSigma now provides ultrasensitive SMC™ immunoassays (developed by Singulex, Inc.) for use on the Erenna® System.

Verified Immunoassay Kits

| Analyte | Assay Format | LLOQ (pg/mL) | Median Endogenous (pg/mL) | Species* | Sample Type [†] | Catalog No. |
|---|--------------|--------------|---------------------------|-----------------|--------------------------|-------------|
| KIM-1 | PBA | 3.91 | P: 65; S: 75; U: 147 | H | P, S, U | 03-0118-00 |
| Akt1 (Ser473) | BBA | 0.98 | NA | H, M, R | L | 03-0100-01 |
| AKT1 (total) | BBA | 7.8 | NA | H, M, R | L | 03-0099-01 |
| Amyloid beta 1-40 | BBA | Coming Soon | | H, M, R | C, P | 03-0155-00 |
| Amyloid beta 1-42 | BBA | Coming Soon | | H, M, R | C, P | 03-0156-00 |
| cTnI | BBA | 0.35 | 1.75 | H, Cy, R, C, GP | P, S | 03-0092-00 |
| cTnI  | BBA | 0.35 | 1.75 | H, Cy, R, C, GP | P, S | 03-0147-00 |
| G-CSF | BBA | 0.08 | 17 | H | P | 03-0047-00 |
| GM-CSF | BBA | 0.02 | 0.2 | H | P | 03-0067-00 |
| GLP-1 (active) | BBA | 0.4 | 3.46 | H, M, R, C | P | 03-0024-03 |
| GLP-1 (total) | BBA | 0.39 | 17.8 | H, M, R, C | P | 03-0025-06 |
| IFN-γ | BBA | 0.2 | 0.79 | H | P | 03-0049-00 |
| IL-1α | BBA | 0.78 | 1.06 | H | P | 03-0072-00 |
| IL-1β  | BBA | 0.1 | 0.08 | H | P, S | 03-0150-00 |
| IL-1β | BBA | 0.2 | 0.08 | H | P | 03-0028-00 |
| IL-2 | BBA | 0.05 | 0.21 | H | P, S | 03-0051-00 |
| IL-4 | BBA | 0.04 | 0.02 | H | P | 03-0052-00 |
| IL-5 | BBA | 3.91 | 4.52 | H | P | 03-0053-00 |
| IL-6 | BBA | 0.08 | 0.01 | H | P, S | 03-0089-01 |

*Optimized for the first species type listed. Other listed species have been tested, but not optimized for peak performance.
KEY: H = human; M = mouse; R = rat; GP = guinea pig; Cy = cynomolgus monkey; C = canine





[†]Optimized for use in sample type(s) listed.

KEY: P = EDTA plasma; S = serum; L = lysate; U = urine; C = cerebrospinal fluid (CSF)



= New easier-to-use protocol

Verified Immunoassay Kits

| Analyte | Assay Format | LLOQ (pg/mL) | Median Endogenous (pg/mL) | Species* | Sample Type [†] | Catalog No. |
|--|--------------|--------------|---------------------------|----------|--------------------------|-------------|
| IL-6  | BBA | 0.08 | 1.3 | H | P | 03-0148-00 |
| IL-7 | BBA | 0.39 | 4.91 | H | P, S | 03-0094-00 |
| IL-8/CX CL8 | BBA | 0.24 | 3.6 | H | P, S | 03-0055-00 |
| IL-10 | BBA | 0.39 | 1.01 | H | P | 03-0056-00 |
| IL-12 | BBA | 0.05 | 0.13 | H | P, S | 03-0057-00 |
| IL-13 | BBA | 0.04 | 0.21 | H | P, S | 03-0109-02 |
| IL-15 | BBA | 0.1 | 3.38 | H | P, S | 03-0058-00 |
| IL-17A | BBA | 0.03 | 0.12 | H | P, S | 03-0103-00 |
| IL-17A  | BBA | 0.02 | 0.12 | H | P, S | 03-0152-00 |
| IL-17F | BBA | 0.2 | 0.86 | H | P, S | 03-0102-00 |
| IL-17F  | BBA | 0.15 | 0.86 | H | P, S | 03-0149-00 |
| IL-17A/F Heterodimer (V2) | BBA | 1.2 | 2.75 | H | P, S | 03-0119-00 |
| IL-21 | BBA | 0.2 | 0.53 | H | S | 03-0014-07 |
| IL-22 | BBA | 0.2 | 3.3 | H | P | 03-0059-01 |
| IL-23 | BBA | 0.1 | 0.18 | H | P, S | 03-0112-00 |
| TNF-α | BBA | 0.02 | 2.3 | H | P | 03-0088-00 |
| TNF-α  | BBA | 0.04 | 2.3 | H | P, S | 03-0151-00 |
| TNF-α | BBA | 0.4 | 38.6 | M | S | 03-0108-00 |
| VEGF | BBA | 0.2 | 66.5 | H | P | 03-0068-00 |

*Optimized for the first species type listed. Other listed species have been tested, but not optimized for peak performance.
KEY: H = human; M = mouse; R = rat; GP = guinea pig; Cy = cynomolgus monkey; C = canine

[†]Optimized for use in sample type(s) listed.

KEY: P = EDTA plasma; S = serum; L = lysate; U = urine; C = cerebrospinal fluid (CSF)



= New easier-to-use protocol

Plate-Based Discovery Immunoassay Kits

| Analyte | LLOQ (pg/mL) | Sample Type | Catalog No. |
|--------------|--------------|-------------|-------------|
| Mouse IL-4 | 0.49 | Serum | 03-0136-00 |
| Mouse IL-5 | 0.24 | Serum | 03-0132-00 |
| Mouse IL-10 | 6.2 | Serum | 03-0134-00 |
| Mouse IL-13 | 3.91 | Serum | 03-0133-00 |
| Mouse IL-17A | 0.98 | Serum | 03-0123-00 |
| Mouse IL-17F | 0.49 | Serum | 03-0125-01 |
| Mouse IL-21 | 4.9 | Serum | 03-0126-00 |
| Mouse IL-22 | 100 | Serum | 03-0127-00 |
| Mouse TNF-α | 0.49 | Serum | 03-0137-00 |

Discovery Immunoassays are provided as complete kits to run on your Erenna® platform.

- Low-Cost
- Simple Workflow
- Reproducible LLOQ
- Minimal Sample Volume

Ongoing Biomarker Research

MilliporeSigma has worked with a number of other biomarkers for use in research programs, demonstrating the versatility of the platform across a variety of disease areas. Contact us to see how you may accelerate your own research in these disease areas.

Prototype Erenna® SMC™ Immunoassays

Neurology

- Aβ-40
- Aβ-42
- VILIP-1

Inflammation

- Mouse IL-2
- Mouse IL-4
- Mouse IL-5
- Mouse IL-10
- Mouse IL-13
- Mouse VEGF
- Mouse TNF-α
- Mouse IFN-γ

Metabolism

- Glucagon
- Insulin
- Active GLP-1 (Plate)
- Total GLP-1 (Plate)

Cardiovascular

- Endothelin

Toxicology

- Skeletal TnI (fast)
- Skeletal TnI (slow)
- NGAL
- Cystatin C

Oncology

- EGFR
- MMP2
- MMP2/TIMP2
- TIMP-2
- tJNK1
- pJNK1
- tJNK2
- pJNK2
- tERK2
- pERK2
- tGSK3β
- pGSK3β

Immunoassay Development Kits

| Development Kit | Catalog No. |
|--|-------------|
| Erenna® Detection Antibody Labeling Kit (for Plate and Bead Based Assay Development) | 03-0076-02 |
| Erenna® Capture Antibody Labeling Kit (for Bead Based Assay Development) | 03-0077-02 |
| Erenna® Bead Based Immunoassay Development Kit | 03-0078-00 |
| Erenna® Plate Based Immunoassay Development Kit | 03-0128-00 |
| Erenna® Assay Buffer Optimization Kit (for Bead Based Assay Optimization) | 03-0122-00 |

EXPERT CUSTOM SERVICES & SAMPLE TESTING

Custom Services and Sample Testing are available when time and resources require an expert outsource partner to help accelerate programs from discovery into clinical trials.

Our capabilities include:

Collaborative Biomarker Discovery & Development

- Fit-for-Purpose Sample Testing (non-GLP)
- Immunoassay Development
- Biomarker Validation (to sponsor requirements)

Biomarker Assay Services for Biotherapeutic Discovery

- Pharmacokinetics
- Pharmacodynamics
- Immunogenicity

Antibody Derivatization/Labeling and Characterization

- Antibody Screening and Selection
- Antibody Labeling
- Assay Stability Testing

SMC™ Assay Kit Development

- Proprietary or Commercial SMC™ Assay Kit Development
- Assay Kit Verification and Validation
- Assay Kit Manufacturing
- Method Transfer to CRO

Sample Testing

- R&D and Prototype Assay Kits
- Commercial Assay Kits
- Potential Biological Relevance in Real Samples
- Biological Anomalies that May Occur Due to Drug Interference, Matrix Effects, etc.

Learn more about our services at:
emdmillipore.com/smctech



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