ELISA/FLISA technique

Enzyme Linked Immuno Sorbent Assay (ELISA) has become a standard immunodetection based technique in most labs. A great advantage is to allow versatile detection in complex biological samples, and indirect quantifications (titration) of biomolecules through measurement of color, fluorescence, or light generated by the enzyme. Now, ELISA and EIA have become generic terms even for non-enzymatic Fluorescence detection systems (FLISA).

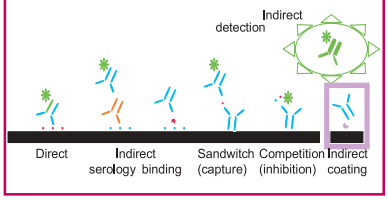
This section presents only reagents dedicated specifically to ELISA/FLISA, including microplates, and some enzymatic substrates. Please refer to other sections for general use reagents (buffers and saturants, I and II antibodies, enzyme substrates,...). Please refer to "Cell biology assay kits" Chapter for antigen-specific ELISA (application kits).

Enzyme Immuno Assay principle

EIA are based 1/ on the immobilization of a primary ligand onto a polystyrene surface (wells of microplates), 2/ on the affinity binding between this ligand and other ligands or antibodies captured from solution phase, 3/on washing of un-reactive compounds, and 4/on the final visualization by a label.

ELISA techniques include several methods (see figure below): the molecule to be detected (in red) may be an antigen ( ) or an antibody ( ) that is coated on microplate (Direct EIA), or captured by affinity of a coated ligand or antibody. The detecting agent is usually an antibody, directly labeled by an enzyme of a fluorophore ( ), that binds the detected molecule without interfering the capture step (Sandwich EIA), or entering in binding competition with it (Competitive EIA).

ELISA/FLISA methods



Microplates (EIA, FPlyte, coated)

Microplates for immunoassays are available in several qualities.

Selection guide

Microplates type	page	Features and applications
High-Bind EIA	page A351	Optimized for Enzyme Immuno Assays
FPlyte cell transparent	page A362	Optimized for Fluorescent Assays . Exists in treated version for culture, and HTS 684 wells format, in black, white or polystyrene
Functionalized / coated plates		Indirect coating for more versatile, quick and reproducible operating with high binding capacity.
avidin plates	pageA364	· for coating of biotinylated abs, peptides or nucleic probes
anti-IgGs plates	pageA364	· for indirect coating of purified or not purified (ascite, sera) I abs
Customized plates		Your specific protocol.

Standard High-Binding EIA Plates

These polystyrene microplates are dedicated to Enzyme Immunoassays (ELISA).

- High capacity or protein coating
- Alphanumeric wells indexation
- Fit standard equipment (128 x 86 mm)
- High optical quality

They are available in complete 96 wells microplates, and 12 strips of 8 wells per plates.

Description	Cat.#	Qty
High-Binding EIA 96 Well Plates	**Q89404**	100 u
	Q89401	5 u
High-Binding EIA 96 Well Strip Plates	**Q89414**	100 u
	Q89411	5 u

Filter Microplates

Standard size 96 wells microplates in which the polystyrene multiwell plate has a multilayered filter instead of a plastic well bottom. The capture antibody or antigen is immobilized onto the filter with higher speed and binding capacity. Following detection steps are preformed by standard procedures, or with electropulsed E3 technology (see page A367).

Description	Cat.#	Qty
E-3® ELISA Plate Assay Kit	**FX6831**	10 u

Related Product

Description	Cat.#	Qty
Coating Buffer concentrate (10 X)	**J0719A**	50 ml

Makes 500 ml of working solution A general purpose ELISA plate coating buffer solution (pH 7.4) suitable for coating most antigens.

ELISA/FLISA technique

FPlyte microplates

Optimized plates are available to suit almost all photometric and cell counting applications :

- Ideal for routine fluorescence, luminescence and scintillation assays
- 24-, 96- and 384-wells formats
- Natural, Tissue culture treated or hight binding treated
- Eliminate misreads and significantly increase precision measurement

FPlyte™ microplates are made of extra-clear polystyrene by advanced technology. They are designed for applications such as fluorescence, luminescence and scintillation in instruments reading from below or above the wells. They are fully compatible with all automated liquid handling systems, photometric readers, and robotic handling devices. Several formats are available, with different sizes, clear or opaque well's walls, with High Bind treated surfaces or Tissue Culture treated surfaces, covering most applications from R&D to QC and HTS. Bar coding is available.

FPlyte™ microplates are used for fluorescence and luminescence applications (reading from below or above). They have respectively black and white opaque matrix limiting individual wells, with a welded clear bottom. They provide highest light transmission.

FPlyte™NCT-B microplates (No Cross Talk) should be used when light cross talk is important to avoid. They have individual wells with individual clear bottoms limited by a ridge that stops any light tranfer to other wells. The result is unmatched reliability and performance.

FPlyte-BW microplates are used for luminescence or scintillation applications with instruments reading from above wells. They have white wells (including the bottom) in a black microplate matrix. The black matrix eliminates totally light crossing between the wells, and the white wells maximize light intensity in luminescences applications. This is supplied with a lid and is individually wrapped.

♦ Size formats :

FPlyte-96 is the popular format, including 96 wells of 350 µl, and compatible with most microplate fluorimeters.

FPlyte-384 is the format dedicated to HTS microplate readers, with 384 wells of 120 µl volume.

FPlyte-24 microplate is a format compatible with the Micro Beta scintillation counter from Perkin Elmer/Wallac, and other specific applications. It offers 24 wells of 3.1 ml volume with large surface area, enabling efficient cell growth.

♦ Treatment formats :

FPlyte-HiBind have wells surface treated for maximal protein binding.

FPlyte-Culture have wells treated for tissue culture (optimized cell attachment and proliferation).

	Nb of wells	Plate matrix	Well walls	Well bottom *	Standard (100u)	FPlyte-HiBind (100u)	FPlyte-Tissue Culture (100u) (with lids, sterile)	FPlyte-TC-HTS (4x20u) (with lids, sterile)
96 wells format microplates								
FPlyte™ 96 x 350µl wells microplates, for fluorescence and luminescence with reading from below or above -standard								
FPlyte-W-96	96	White	White	Clear	**FP-BA7810**	-	**FP-BA7880**	-
FPlyte-B-96	96	Black	Black	Clear	**FP-BA7890**	-	**FP-BA7910**	-
FPlyte™NCT 96 x 350µl wells microplates, for fluorescence and luminescence with reading from below or above – NO light cross talk								
FPlyte-NCTW-96	96	White	White	Clear b	**FP-BA7950**	**FP-BA7960**	**FP-BA7970**	**FP-BA8020**
FPlyte-NCTB-96	96	Black	Black	Clear b	**FP-BA7990**	**FP-BA8000**	**FP-BA8010**	**FP-BA8030**
FPlyte-BW 96 x 350µl wells microplates, for luminescence with reading from above								
FPlyte-BW96	96	Black	White	White	**FP-BA8040**	**FP-BA8050**	**FP-BA8060**	-
384 wells format microplates								
FPlyte 384 x 120µl wells microplates, for fluorescence and luminescence with reading from below or above - standard								
FPlyte-C384	384	Clear	Clear	Clear	**FP-BA8210**	**FP-BA8220**	**FP-BA8230**	-
FPlyte-W384	384	White	White	Clear	**FP-BA8240**	**FP-BA8250**	**FP-BA8280**	-
FPlyte-B384	384	Black	Black	Clear	**FP-BA8290**	**FP-BA8300**	**FP-BA8320**	-
FPlyte-SV 384 x 30µl wells microplates,for fluorescence and luminescence with reading from below or above – small volume sample (30µl)								
FPlyte-C384sv	384	Clear	Clear	Clear	**FP-BB1370**	-	**FP-BB1400**	-
FPlyte-W384sv	384	White	White	Clear	**FP-BB1410**	-	**FP-BB1440**	-
FPlyte-B384sv	384	Black	Black	Clear	**FP-BB1450**	-	**FP-BB1480**	-
FPlyte-NCT 384 x 120µl wells microplates, for fluorescence and luminescence with reading from below or above – Minimal light cross talk								
FPlyte-NCTW 384	384	White	White	Clear bt	**FP-BA8130**	-	**FP-BA8160**	**FP-BA8190**
FPlyte-NCTB 384	384	Black	Black	Clear bt	**FP-BA8170**	-	**FP-BA8180**	**FP-BA8200**
384 wells samples **Storage microplates,** for sample storage (solvent resistant polypropylene)								
Storage384 well plates	384	PP	PP	PP	**FP-BA8340**	-	-	-
24 wells format microplates								
FPlyte-BW 24x3.1ml wells microplates,for large volumes/cell culture + fluorescence and luminescence with reading from below or above								
FPlyte-W24	24	White	White	Clear b	**FP-BA8070**	**FP-BA8080**	**FP-BA8090**	-
FPlyte-B24	24	Black	Black	Clear b	**FP-BA8100**	**FP-BA8110**	**FP-BA8120**	-

*Clear b : clear individual bottom ; Clear bt : thin clear bottom ; PP : PolyPropylene

ELISA/FLISA technique

Streptavidin coated microplates

Ideal for indirect coating of biotinylated ligands (Abs, peptides, DNA/RNA probes...)

Contrary to passive coating, which can be affected by ionic probes or hydrophobic characteristics, streptavidin-biotin system offers a more homogeneous coating which can be very useful for the screening of probes from libraries.

- Easy and consistent coating
- Highest ratio Signal to noise
- Ready-to-use (pre-saturated)

Streptavidin coated plates provide a universal support to fastly coat biotinylated molecules for immunoassays. However, home made avidin coating is usually poorly consistent because of (strep)avidin sensitivity to drying/storage conditions once coated. Supplied pre-blocked with Seablock agent (UP40301) with an optimized protocol, our streptavidin plates improve signal to noise ratios and suit both colorimetric and chemiluminescent detections.

Description	Cat.#	Qty
Streptavidin coated microplates	**UPL76161**	5 plates

See also CultureWell® chambered coverglass/ELISA (Elispot slides) in section IHC.

Anti IgG (Gt) coated microplates

Ideal for indirect coating of purified or not purified (ascite, sera) I abs

- Quicker to use
- Lowest background
- Higher and reproducible results

Our plates use Fc-oriented coating and highest affinity anti IgGs for optimal IgG binding capacity (superior to passive direct coating of your I Ab, and quicker). These plates are supplied pre-blocked with Seablock agent (UP40301), to save your time and give excellent results in both colorimetric and chemiluminescence detections.

Description	Cat.#	Qty
Anti Mouse IgG (Gt) coated microplates	**UP47246A**	5 plates
Anti Rabbit IgG (Gt) coated microplates	**UP42458A**	5 plates

Custom microplates coating

Ask us to coat your antigens according our standard procedures, or according your specific requirements (saturant) or protocol. See section 'Custom services'. Saved time and money to better focus on other essentials tasks !

Fluorescence Assay Calibration Kit *For Microplate reader or spectrophotometer*

They are used for calibrating fluorescence instruments. Each kit contain a set of stable and water-soluble dyes to cover the full fluorescence spectrum. All the dyes have similar fluorescence quantum yields and photostabilities in water or aqueous buffer.

Description	Application	Cat.#	Qty
AnaStandard™ 350/430	AMC, AFC or AMCA or other coumarin-based assays	**BS5870**	1 kit
AnaStandard™ 510/530	Fluorescein, FAM, FITC or Rh110-based assays	**BS5880**	1 kit
AnaStandard™ 550/570	Cy3, TAMRA, sulforhodamine and rhodamine B-based assay	**BS5890**	1 kit
AnaStandard™ 600/630	ROX, Sulforho-damine B or Sulforhodamine 101 based assays	**BS5900**	1 kit
AnaStandard™ 650/670	Cy5-based assays	**BS5910**	1 kit
AnaStandard™ *Full Spectrum*	Calibrating fluorescence instruments	**BS5920**	1 kit

ELISA chromogenic substrates

Peroxidase (HRP) conjugates are extensively used as secondary detection reagents in ELISAs, immuno-histochemical techniques, Northern, Southern and Western blot analyses.
Alkaline phosphatase (AP) conjugates widely used in various biological assays including ELISAs, immunohistochemical techniques and Western blot analyses.
Following are effective ready to use substrates for colorimetric and fluorometric detections in EIA.

Uptima TMB

Solutions are optimized chromogenic substrates for peroxidase, designed for ELISA techniques, manual or automatic systems. They contain 3,3',5,5'-tetramethylbenzidine (TMB), hydrogene peroxide (H_2O_2), and proprietary catalyzing and stabilizing agents.

- Highest sensitivity
- Reproducible lots
- Ready-to-use

Description	Cat.#	Qty
TMB, "Standard" solution	**UP664780**	200 ml
The original formulation, ideal for classic applications	**UP664781**	500 ml
Highest sensitivity	**UP664782**	1 l
Stability > 24 months at +4°C		
TMB, "Check" solution	**UPS08170**	100 ml
Recommended for routine assays, to better control	**UPS08171**	500 ml
failure in reagent distribution.		
Includes a red dye that do not interfere with reaction nor final reading		
High sensitivity ; Stability > 1 year at +4°C		
TMB, "Aqueous" solution	**UPS08180**	100 ml
Recommended in particular for diagnostic kits	**UPS08181**	200 ml
Non-hazardous, non-volatile, non-organic, non-toxic	**UPS08182**	500 ml
(does not contain DMF, DMSO) :	**UPS08183**	1 l
100% water based formulation to maximize the safety (no regulation concerns).		
Highest sensitivity ; Stability > 1 year at +4°C		

Related product

To be used with TMB reagents
Stops the reaction, change the color to deep yellow, increasing the sensitivity 2 to 3 fold.

Description	Cat.#	Qty
TMB / HRP Stop solution	**UPS29590**	250 ml

pNPP (p-Nitrophenyl Phosphate)

The preferred substrate for high sensitivity detection of alkaline phosphatase in EIA assays. It produces a yellow product that absorbs at 405 nm.

Description	Cat.#	Qty
pNPP 5/175 mg tablets	**UP89562G**	100 tabs
	UP89562F	1000 tabs
each tablet contains 5 mg of pNPP for quick and easy preparation of substrate solution.		
pNPP 30/175 mg tablets	**UP732500**	100 tabs
	UP732501	1000 tabs
each tablet contains 30 mg of pNPP for quick and easy preparation of substrate solution.		
PNPP powder	**UP89562C**	25 g
	UP89562D	100 g

pNPP Alkaline Phosphatase ELISA Assay Kit *Colorimetric*

Alkaline phosphatase (AP) is a popular enzyme conjugated with secondary antibody for ELISA. p-Nitrophenyl phosphate (pNPP) is proven to be an effective chromogenic substrate for alkaline phosphates. The EnzoLyte™ pNPP Alkaline Phosphatase ELISA Assay Kit provides optimized pNPP assay buffer, modified ELISA wash buffer and AP-conjugated secondary antibody. The assay is highly sensitive and has a large dynamic range. The kit contains: pNPP chromogenic substrate (λ_{max} = 405 nm upon dephosphorylation), Assay buffer, ELISA wash buffer, Stop solution, Alkaline phosphate-conjugated secondary antibody (goat anti-rabbit or goat anti-mouse IgG), an optimized ELISA assay protocol.

Description	Cat.#	Qty
pNPP Alkaline Phosphatase ELISA Assay Kit	**BP7080**	500 assays

ELISA/FLISA technique

FLISA Fluorogenic substrates

ADHP Peroxidase ELISA Assay Kit "Fluorimetric"

EnzoLyte™ ADHP Peroxidase ELISA Assay Kit uses highly purified ADHP to quantify HRP activity and detect as low as femto molar (10^{-15} M) concentration of primary antibodies in an ELISA format. ADHP (10-Acetyl-3, 7-dihydroxyphenoxazine) is oxidized by H_2O_2 catalyzed by HRP to produce a fluorescent product resorufin. The kit contains : ADHP peroxidase substrate ($\lambda_{abs}/\lambda_{em}$: 573/590 nm upon oxidation), Reaction buffer, Stop buffer, and HRP-conjugated goat anti-rabbit IgG.

Description	Cat.#	Qty
ADHP Peroxidase ELISA Assay Kit "Fluorimetric"	**HS6240**	500 assays

FDP Alkaline Phosphatase ELISA Assay Kit "Fluorimetric"

EnzoLyte™ FDP Alkaline Phosphatase ELISA Assay Kit uses highly purified FDP to quantify alkaline phosphatase activity. It has been used to detect as low as atto molar (10^{-18} M) concentration of primary antibodies in an ELISA format. The kit contains FDP phosphatase substrate ($\lambda_{abs}/\lambda_{em}$: 485 ±20/528 ±20 nm upon dephosphorylation), Assay buffer, Stop solution, Wash buffer, and AP-conjugated secondary antibody (goat anti-rabbit or goat anti-mouse IgG).

Description	Cat.#	Qty
FDP Alkaline Phosphatase ELISA Assay Kit "Fluorimetric"	**HT0790**	1000 assays

Technical tip - Microplate readers

Interchim and Berthold collaboration supports further your works. Many of our fluorescence and luminescence reagents and kits were validated with 'BERTHOLD TECH Log microplate readers.

***Mithras LB940 MultiMode Reader**

Includes a variety of technologies with samples injectors and robot integration module.

- Various formats (from Petri dishes to 1536 well plates)
- Absorbance
- Luminescence
- Fluorescence
- top and bottom measurement
- Polarisation (FP)
- FRET
- BRET
- AlphaScreen™
- High density and normal TRFluorometry

***Centro LB960 Luminometer**

a robust, versatile and sensitive microplate luminometer (lowest crosstalk) exists also in a Clinical version (LB961).

***Twinkle LB970 Fluorometer**

Reading from above and below, from Petri dishes to 864 well microplates. Ideal for sensitive FRET assays

***Apollo LB911 Absorbance Readers**

12-96 wells or 96-384 wells microplate in 400-800nm range, with single channel

***Apollo LB92 Absorbance Reader**

96 wells microplate in 340-800nm range, with 8-channels

E3 ELISA System

E3® technology use patented programmed electropulsing to accelerate biomolecule complex formation, and standard size 96 well plates in which the polystyrene has a multilayered filter instead of a plastic well bottom. As a result, E3® ELISA system dramatically reduces the time required for incubation periods compared to standard ELISA procedures : an ELISA can be completed in only 30 minutes !

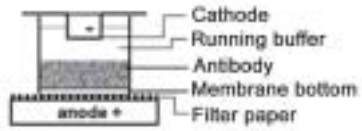
- Increases reproducibility by totally controlling immunoassay conditions
- Increases signal intensity and sensitivity
- Save on reagent quantity (50 % less)

Description	Cat.#	Qty
E-3® ELISA Apparatus	**FX6701**	1 u
Utilized standard size 96 wells filter plates. The bottom flat anode contacts each well via the wet filter. The upper cathode individually protrude in each well.		
E-3® Power Switch (Power supply)	**BP2721**	1 u
Power supply for electrophoresis, blotting transfer, and E-3 immunodetection procedures.		
E-3® ELISA Plate Assay Kit	**FX6830**	10 u

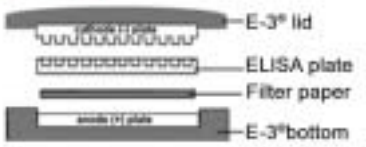
Contains : single components are available separately as :

96-well plates plates	**FX6731**	10 u	**FX6730**	2 u
Filter Paper sheets	**FX6741**	40 u	**FX6740**	8 u
5X Running buffer	**FX6751**	500 ml	**FX6750**	120 ml
Blocking buffer	**FX6761**	250 ml	**FX6760**	70 ml
10X Wash/Coating buffer	**FX6771**	200 ml	**FX6770**	50 ml
Chemi-Substrate	**FX6811**	25 ml	**FX6810**	5 ml
Loading Buffer 200 ml	**FX6781**	200 ml	**FX6780**	50 ml

Single Well View

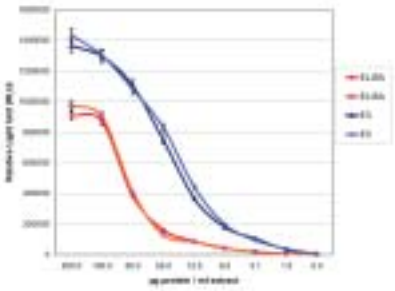


Assembly



Direct ELISA for detection of G3PGH from Jurkat cell line

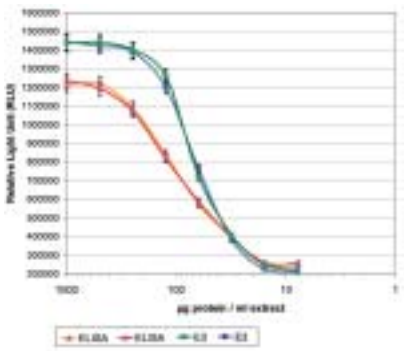
	Standard ELISA	E3® ELISA
Jurkat cells extract coating	1 H	5 min
Blocking	1 H	5 min
Ag capture RT	1 H	6 min
II Ab detection	1 H	6 min
Total procedure:	**~ 4H30**	**~ 30 min**



63P6H Expresion cell extract.

Capture assay for detection of EBV VCA antigen

	Standard ELISA	E3® ELISA
Anti VCA Ab coating RT	2 H	5 min
Blocking	1 H	5 min
Antigen capture	2 H	5 min
biotin-anti VCA Ab capture RT	1 H	6 min
Streptavidin-HRP detection	20 min	6 min
Total procedure:	**~ 7 H**	**~ 30-50 min**



Capture assay for detection of EBV VCA antigen