

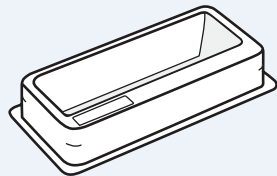
ELISA Workflow Guide

OVERVIEW

Enzyme Linked Immunosorbent Assay (ELISA) is accurate, highly sensitive, and specific for identifying protein species. ELISA microplates enable a common laboratory procedure to be carried out on multiple samples simultaneously. Popular formats include 96-well microplates, 384-well microplates, and 8-well strips.

This guide provides an overview of the tools you'll need at each stage of the ELISA workflow, as well as a few tips for choosing the optimal microplate for your particular assay. Corning is a leading manufacturer of high quality, high performance ELISA microplates and 1 x 8 Corning® Stripwell™ microplates for a wide range of laboratory assays. Corning also carries an extensive variety of accessories that can be used as part of the ELISA workflow, including a full line of buffers, pipets, tips, and tubes to meet unique assay needs.

Reservoirs



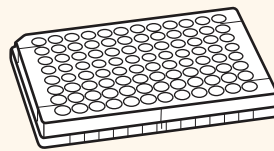
Costar® Reagent Reservoirs

- ▶ Use with multi-channel pipets to transfer samples, buffers, or reagents into ELISA microplates

Axygen® Multi-channel Reservoirs

- ▶ Single and multiple well formats for manual and automated platforms
- ▶ Multi-channel reservoirs allow for separation of reagents during ELISA preparation

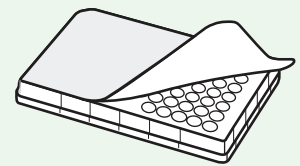
Microplates



Corning/Falcon® Microplates

- ▶ Available in clear, black, or white polystyrene to suit various detection methods: absorbance, fluorescence, or luminescence
- ▶ Medium or High Binding surfaces most commonly used for biochemical assays based on size of target molecule
- ▶ Additional surfaces are available to support other assay types

Microplate Seals



Axygen ELISA Microplate Sealing Films

- ▶ Used during repetitive incubation steps during ELISAs to reduce reagent evaporation, which can cause an "edge effect"

Corning Aluminum Microplate Sealing Tape

- ▶ Utilize during incubation steps to protect light-sensitive samples or reagents for direct and sandwich ELISA

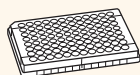
BASIC WORKFLOW

Process Step

Products Used

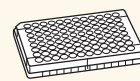
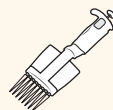
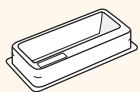
Select the Correct Microplate and Surface for the Application

- ▶ Well format (96-well, 384-well, 8-well strips)
- ▶ Surface chemistry (medium bind, high bind, or other surface type)
- ▶ Microplate color (clear, black, white)



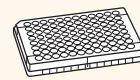
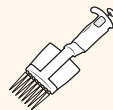
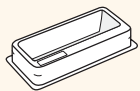
Coat

- ▶ Coat the microplate surface with solution of either antigen or antibody of interest
- ▶ Incubate
- ▶ Wash to remove unbound material



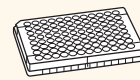
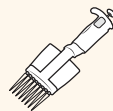
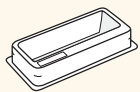
Block (if required)

- ▶ Add protein-based solution to block unbound sites on microplate surface (BSA, Casein)
- ▶ Incubate
- ▶ Wash to remove excess blocking solution (if required)



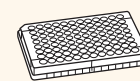
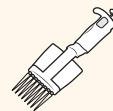
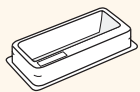
Add Detection Substrate Solution

- ▶ Enzyme, fluorophore-conjugated Ab or direct binding
- ▶ Aluminum sealing tape (ideal for fluorescent reactions)
- ▶ Incubate at the appropriate temperature for the detection method used



Read Signal

- ▶ Add stop solution (if required)
- ▶ Measure produced signal via absorbance, fluorescence, or luminescence



PRODUCTS

Microplates

Color

Clear – Best suited for absorbance detection

Black – Low background fluorescence and low fluorescent cross-talk. The black colorant reduces background, as well as light scattering, resulting in higher signal-to-noise ratios.

White – Enhances luminescence signal-to-noise ratio by reflecting light back into the range of the detector.

Surface Chemistry

Medium Binding Surface

- ▶ Hydrophobic
- ▶ Ideal for large, hydrophobic biomolecules (>20 kD)
- ▶ Binding capacity: ~200 ng IgG/cm²

High Binding Surface

- ▶ Hydrophobic and ionic (negatively charged)
- ▶ Ideal for positively charged biomolecules (>10 kD)
- ▶ Binding capacity: ~500 ng IgG/cm²

Corning 96-well ELISA Microplates

VWR Cat. No.	Corning Cat. No.	Type	Color	Surface	Qty/Pk	Qty/Cs
29442-316	3591	Flat-bottom	Clear	Medium binding	1	50
29442-314	3590	Flat-bottom	Clear	High binding	1	100
29442-070	9017	Flat-bottom	Clear	Medium binding	25	100
29442-322	9018	Flat-bottom	Clear	High binding	25	100
29444-016	3912	Flat-bottom	White	Medium binding	25	100
29444-020	3922	Flat-bottom	White	High binding	25	100
29444-018	3915	Flat-bottom	Black	Medium binding	25	100
29444-022	3925	Flat-bottom	Black	High binding	25	100

Falcon® 96-well ELISA Microplates

VWR Cat. No.	Corning Cat. No.	Type	Color	Surface	Qty/Pk	Qty/Cs
15705-066	351172	Flat-bottom, with lid	Clear	Not treated	1	50
12777-030	351190	Round-bottom	Clear	Not treated	25	100

Corning Stripwell™ 96-well ELISA Microplates

VWR Cat. No.	Corning Cat. No.	Type	Color	Surface	Qty/Pk	Qty/Cs
29442-304	2593	Flat-bottom	Clear	Medium binding	25	100
29442-302	2592	Flat-bottom	Clear	High binding	25	100
29444-028	3923	Flat-bottom	White	High binding	25	100
29444-030	3924	Flat-bottom	Black	High binding	25	100

Corning 384-well ELISA Microplates

VWR Cat. No.	Corning Cat. No.	Type	Color	Surface	Qty/Pk	Qty/Cs
29444-096	3700	Flat-bottom	Clear	High binding	25	100
29444-092	3702	Flat-bottom	Clear	Not treated	25	100
89089-794	3576	Flat-bottom	White	High binding	10	50
89089-792	3572	Flat-bottom	White	Not treated	10	50
89089-782	3577	Flat-bottom	Black	High binding	10	50
89089-788	3573	Flat-bottom	Black	Not treated	10	50

Costar® Reagent Reservoirs

- ▶ Manufactured from modified polystyrene
- ▶ Sterile
- ▶ Disposable
- ▶ 50 mL or 100 mL volume

VWR Cat. No.	Corning Cat. No.	Volume	Color	Qty/Pk	Qty/Cs
53504-035	4870	50 mL	White	5	200
29442-476	4872	100 mL	White	5	200

Axygen® Single- and Multi-channel Reservoirs

- ▶ Complies with ANSI/SLAS footprint for automation
- ▶ Multi-channels allow separation of multiple assays and buffers during ELISA
- ▶ Ranges from 1- to 12-channel reservoirs

VWR Cat. No.	Corning Cat. No.	Channel	Volume/ Color	Total Channel	Volume	Qty/Pk	Qty/Cs
47743-958	RES-SW96-HP	Single	Clear	240 mL	240 mL	25	25
89179-656	RES-MW4-HP	Four	Clear	70 mL	280 mL	25	25

Axygen ELISA Microplate Sealing Film

- ▶ Polyester-based with uniformly applied acrylic adhesive that reduces the edge effect of sensitive ELISA assays
- ▶ Suitable for short-term storage and incubation
- ▶ Utilized for incubation of ELISA assays and buffers

VWR Cat. No.	Corning Cat. No.	Material	Thickness	Dimension (mm)	Working Temp.	Qty/Pk	Qty/Cs
10011-117	PCR-SP	Polyester	80 µm	146 x 79.6	104°C	100	500

Corning® Aluminum Microplate Sealing Tape

For light sensitive samples and reagents

VWR Cat. No.	Corning Cat. No.	Material	Microplate	Qty/Pk	Qty/Cs
29445-080	6570	Aluminum	96-well	100	100
29445-082	6569	Aluminum	384-well	100	100

ELISA Technical Documents


Five ELISA Application Notes are available:

- ▶ Immobilization Principles – Selecting the Surface for ELISA Assays
- ▶ Optimizing the Immobilization of Protein and Other Biomolecules for ELISA Assays
- ▶ Effective Blocking Procedures in ELISA Assays
- ▶ Optimizing the Separation Step on 96-well Microplates for ELISA Assays
- ▶ Selecting the Detection System - Colorimetric, Fluorescent, Luminescent Methods for ELISA Assays

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