



**ASSAYS IN A FLASH
FAST & EASY KIT DEVELOPMENT**

1.0 INTRODUCTION

Intended use: Develop your own assays with proven commercial reagents known worldwide for performance and stability. Direct application to plate (96 well x 10) of antibody (antigen) in fast and easy procedure (color-coated to avoid missed-wells), followed by overnight incubation and two washes.

Get your assay running: starting antibody (antigen) concentration given with no expensive equipment required and batch-to-batch consistency.

For Research Use Only

2.0 KIT OPTIONS

A. AccuFlash™ ELISA Microwells

Small Molecule <i>Typically <1,000 MW & Competitive Assay</i>	ITEM#	AF-EIA-SM-10
Peptides or Proteins <i>Typically > 5,000 MW & Sandwich Assay</i>	ITEM#	AF-EIA-PP-10

B. AccuFlash™ CLIA Microwells

Small Molecule <i>Typically <1,000 MW & Competitive Assay</i>	ITEM#	AF-CIA-SM-10
Peptides or Proteins <i>Typically > 5,000 MW & Sandwich Assay</i>	ITEM#	AF-CIA-PP-10

3.0 REAGENTS

ELISA MATERIAL PROVIDED:

- A. Activated ELISA coated plates – 96 wells – Icon** ↓
Ten 96-well activated ELISA microplates packaged individually in reusable aluminum bag with a drying agent. Store at 2-8°C.
- B. Desiccant**
Ten drying agents for plate storage after coating with antibody.
- C. Coating Buffer with Die (blue) – 125ml**
Buffer for diluting biotinylated antibody. A blue dye has been added. Store at 2-8°C.
- D. Stabilization Buffer – 1000ml**
Buffer for stabilizing plate after antibody incubation. Store at 2-8°C.
- E. HRP Conjugate Diluent**
 - 1. **Small Molecule HRP Conjugate Diluent - 125ml**
HRP Conjugate for small molecules with blockers for heterophilic antibodies. Store at 2-8°C.
 - OR**
 - 2. **Peptides or Proteins HRP Conjugate Diluent - 125ml**
HRP Conjugate for peptides or proteins with blockers for heterophilic antibodies. Store at 2-8°C.
- F. TMB Substrate – 52 ml/vial- Icon S^N**
One (1) bottle contains tetramethylbenzidine (TMB) and hydrogen peroxide (H₂O₂) in buffer. Store at 2-8°C.
- G. Wash Solution Concentrate – 60ml - Icon** ⚠
One (1) vial contains surfactant in buffered saline. A preservative has been added. Store at 2-30°C.
- H. Stop Solution -- 30ml/vial - Icon** ⛔
One (1) vial contains a strong acid. Store at 2-30°C.

I. Product Instructions

CLIA MATERIAL PROVIDED

- A. Activated CLIA coated plates Plate – 96 wells – Icon** ↓
Ten 96-well activated CLIA microplates packaged individually in aluminum bag with a drying agent. Store at 2-8°C.
- B. Desiccant**
Ten drying agents for plate storage after coating with antibody.
- C. Coating Buffer with Die (blue) – 125ml**
Buffer for diluting biotinylated antibody. A blue dye has been added. Store at 2-8°C.
- D. Stabilization Buffer – 1000ml**
Buffer for stabilizing plate after antibody incubation. Store at 2-8°C.
- E. HRP Conjugate Diluent**
 - 1. **Small Molecule HRP Conjugate Diluent - 125ml**
HRP Conjugate for small molecules with blockers for heterophilic antibodies. Store at 2-8°C.
 - OR**
 - 2. **Peptides or Proteins HRP Conjugate Diluent - 125ml**
HRP Conjugate for peptides or proteins with blockers for heterophilic antibodies. Store at 2-8°C.
- F. Signal Reagent A – 30 ml/vial- Icon CA**
One (1) bottle containing luminol in buffer. Store at 2-8°C.
- G. Signal Reagent B – 30ml/vial - Icon CB**
One (1) bottle containing hydrogen peroxide (H₂O₂) in buffer. Store at 2-30°C.

- H. Wash Solution Concentrate – 60ml - Icon** ⚠
One (1) vial contains surfactant in buffered saline. A preservative has been added. Store at 2-30°C.

4.0 MATERIALS REQUIRED BUT NOT PROVIDED:

- 1) Biotinylated antibody¹ or antigen for coating
- 2) HRP incorporation¹ of antibody or antigen
- 3) 8-channel Multipipette – Optional
- 4) Automatic Washer – Optional Monobind Plate Washer
Recommended Monobind ITEM# IN002
- 5) One plate cover per 10 plates

¹Thermofisher has biotinylation and HRP incorporation procedures that are direct and easy to implement. Express antibody or antigen concentrations in mass units (µg/ml).

5.0 REAGENT PREPARATION:

1. **Wash Buffer**
Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at room temperature (20-27°C) for up to 60 days.

6.0 PROCEDURE:

- 1) Dilute biotinylated antibody to a suggested target concentration of 1µg/ml in coating buffer (blue color).
- 2) Open activated plate(s), remove from bag and retain bag for later storage of coated plates
- 3) Add 100µl diluted biotinylated antibody to each well using a pipette. An 8-channel pipette is recommended when coating a large number of plates. Stack plates on top of each other. Hint: It is not necessary to cover each plate except the top plate.
- 4) Incubate overnight at room temperature.
- 5) Wash each plate using the following sequence. Double aspiration, dispensed 350µl stabilization buffer, double aspiration, dispensed 350µl stabilized buffer and final double aspirate. Note: final aspiration should remove virtually all residual moisture
- 6) Invert (and tap) plates onto a paper towel. Let dry in open air for 4 hours or place in 37°C oven for 1 hours.
- 7) Place plate in original foil bag (or comparable closure) along with a new drying agent and seal package with the reusable zip closure.

7.0 INITIAL ASSAY DEVELOPMENT PROCEDURE:

- 1) Add 25µl or 50µl of calibrators or sample² to a microwell.
- 2) Add 50µl or 100µl of appropriate HRP conjugate diluent (start at 50ng/ml for antibody or 5ng/ml for antigens).
- 3) Incubate for 1 hour at room temperature with or without shaking³.
- 4) Wash 3 times using double aspirate, 350µl wash buffer with a final double aspirate.
- 5) Add 100µl TMB Substrate or Signal Reagent⁴.
- 6) Incubate 15-20 minutes with TMB Substrate or 5 minutes with Signal⁵.
- 7) Add 50µl Stop Solution for ELISA
- 8) Read the absorbance for ELISA or measure light for CLIA.

²The calibrator concentrations should be determined by relevant levels required by developer. Stability of calibrators need to be established by appropriate stability protocols. The Protein matrix that is included has proven to be of general use for peptides or proteins. The small molecule matrix is processed human serum that

has most small molecules removed (such as steroids etc.). Other matrixes are available upon request.

³Faster reaction occurs with shaking if no shaking does not provide the desired color or light signal.

⁴Signal reagent can be combined for one addition or each reagent can be dispensed independently at 50µl each per well.

⁵No stop solution is added for signal

8.0 ESTABLISH SPECIFIC PROTOCOL

- 1) Adjust (Titer) concentration of HRP reagent to provide desired response. Normally the antibody coating conditions does not need to be changed. However, one could experiment with this as well.
- 2) Change incubation parameters for optimum conditions (such as time, shaking etc.)..
- 3) Different calibrator matrixes can be used. Monobind has a large family of protein matrixes available.

9.0 VALIDATE ASSAY

Use CLSI standards to validate or acceptable assay performance specifications.

TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

E-mail at info@monobind.com or technicalsupport@monobind.com

CALIBRATOR MATRIXES



Proven globally in commercial systems, Monobind's biomatrixes are used in its own product lines including AccuBind® ELISA, AccuLite® CLIA, and QSure® Controls, as well as in other manufacturer's methods.

Monobind has honed proprietary methods to produce high-quality biomatrixes, for use with small and large proteins (stable or labile) and small molecules, including drugs. For selection of available matrixes (in minimum volumes of 125ml), see <http://www.monobind.com/biomaterials> and contact technicalsupport@monobind.com for further assistance.

STABILIZING BUFFERS

ActiveZyme® is an HRP-based buffer developed by Monobind to support long-range enzyme-stability for its entire immunoassay line. Measurement of absorbance (ELISA) or RLU (CLIA) remain consistent over time, ensuring more accurate reporting of analyte concentration. Real-time comparative studies demonstrate the superior performance of ActiveZyme compared to other commercial stabilizer products.

ActiveZyme allows development of ready-to-use enzyme, as well as serves as an effective preservative improving transport stability. Please see <http://www.monobind.com/r-d-immunoassay-tools-conjugate-stabilizer> for more details and contact sales@monobind.com for further assistance.



INSTRUMENTS

Monobind offers a line of laboratory instruments, including automated plate washers which assist in procedure of AccuFlash, as well as easily programmed ELISA or CLIA strip and plate readers.

Please see <http://www.monobind.com/instruments> for more details.



For Orders and Inquiries, please contact



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