Conversion to MicroFluere® from Traditional Well Plate Using R&D Systems's DuoSet ELISA Kits

Note:

Please do read: MicroFluere ELISA Tips

Please do watch:
Pipetting video
Pipetting2 video
Pipetting Standard video
Reservoir video
Standard Curve video
Drainage Device video

Buffers

The buffers can be used as the same as described in the DuoSet ELISA Kit protocol.

1. **Coating buffer (PBS)**

   PBS ELISA Plate-coating Buffer catalogue # DY006 from R&D Systems

   https://www.rndsystems.com/products/elisa-plate-coating-buffer_dy006
2. **Blocking buffer, sample, and reagent diluent (1%BSA)**

   Reagent Diluent Concentrate 2 catalogue Catalog # DY995 from R&D Systems

   Diluted 10 times in DI water for 1x working concentration.

   https://www.rndsystems.com/products/reagent-diluent-concentrate-2_dy995
3. **Washing buffer**

   Quantikine ELISA Wash Buffer 1, Catalog # WA126

   Diluted 25 times in DI water for 1x working concentration.

   https://www.rndsystems.com/products/quantikine-elisa-wash-buffer-1_wa126

Substrate

MicroFluere® has been tested and compatible with following substrate.

1. **Substrate**

   QuantaRed™ Enhanced Chemifluorescent HRP Substrate Kit (Catalog number: 15159)

   https://www.thermofisher.com/order/catalog/product/15159#/15159

Conversion of working concentrations of reagents for MicroFluere®

A general conversation of reagent concentrations for MicroFluere® is tabulated below.

Reagent	Concentration- recommended (range)
Capture	2x concentration of traditional well plate (2 to 4x) e.g., 4 μg/mL is used for MicroFluere® if 2 μg/mL is recommended for traditional well plate.
Detection	1x concentration of traditional well plate (1 to 1.5x)
Streptavidin-HRP	1.5x concentration of traditional well plate (1.2 to 1.8x)

Example: Human IL-6 (DY206) ELISA Protocol

Stock Solution (https://resources.rndsystems.com/pdfs/datasheets/dy206.pdf)

First, stock solutions are prepared as described in the product manual (example below).

REAGENT PREPARATION Bring all reagents to room temperature before use. Allow all components to sit for a minimum of 15 minutes with gentle agitation after initial reconstitution. Working dilutions should be prepared and used immediately.

Streptavidin-HRP: Each vial contains 2.0 mL of streptavidin conjugated to horseradish-peroxidase. Dilute to the working concentration specified on the vial label using Reagent Diluent.

Mouse Anti-Human IL-6 Capture Antibody: Refer to the lot-specific C of A for amount supplied. Reconstitute each vial with 0.5 mL of PBS. Dilute in PBS without carrier protein to the working concentration indicated on the C of A.

Biotinylated Goat Anti-Human IL-6 Detection Antibody: Refer to the lot-specific C of A for amount supplied. Reconstitute each vial with 1.0 mL of Reagent Diluent. Dilute in Reagent Diluent to the working concentration indicated on the C of A.

Recombinant Human IL-6 Standard: Refer to the lot-specific C of A for amount supplied. Reconstitute each vial with 0.5 mL of deionized or distilled water.

Working Solution

Second, working solutions are reconstituted based on certificate of analysis (C of A) provided by kit manufacturer to achieve desired concentrations as described in the conversion table.

An example of C of A of Human IL-6 ELISA kit is shown as below.

Certificate of Analysis

SPECIFICATIONS

REAGENT	PART NUMBER	# OF VIALS	AMOUNT PER VIAL	WORKING CONCENTRATION	LOT #
Capture	840113	3	120 µg	2.00 µg/mL	HD5219101
Detection	840114	3	3.00 µg	50.0 ng/mL	SV2919101
Standard	840115	3	90.0 ng	9.38-600 pg/mL	1515238
Streptavidin-HRP	893975	3	N/A	40-fold dilution	P228155

PREPARATION & STORAGE

Store unopened kit at 2-8 °C. Do not use past kit expiration date.

REAGENT	PREPARATION	STORAGE OF OPENED/RECONSTITUTED MATERIAL
Capture	Reconstitute with 0.5 mL of PBS	Store at 2-8 °C for up to 8 weeks or aliquot and store at -20 °C to -70 °C in a manual defrost freezer for up to 12 weeks.*
Detection	Reconstitute with 1.0 mL of Reagent Diluent	
Standard	Reconstitute with 0.5 mL of deionized or distilled water	Store reconstituted standard at 2-8 °C for up to 8 weeks or aliquot and store at -70 °C for up to 12 weeks.*
Streptavidin-HRP	Dilute with Reagent Diluent	Store undiluted at 2-8 °C for up to 12 weeks. DO NOT FREEZE.*

*Provided this is within the expiration date of the kit.

Protocol

Step	Concentration	Buffer	Volume (µL)	Incubation (min)
Capture	4 µg/ml (60x dilution from stock solution)*	PBS	20	60
Blocking	1x	1%BSA	20	30

After coating with capture and blocking, the MicroFluere® well plate can be stored in cool dry place for future use, or we can immediately follow following steps.

Step	Concentration	Buffer	Volume (µL)	Incubation (min)
Standard/Sample	Start with 9.6 ng/mL (18.75x dilution from stock solution)*, 2-fold serial dilution until 9.375 pg/mL (11 standards)	1%BSA	20	15
Detection	50 ng/ml (60x dilution from stock solution)*	1%BSA	20	15
HRP	1:25 dilution	1%BSA	15	5
Wash 3 times	1x washing buffer (25x dilution from stock solution)*		20	-
Wash 1 time		PBS	20	-
Substrate	--	--	13	5-15

*Dilution factors as described in the above table are based on the example C of A. The C of A may change lot to lot. Therefore, dilution factors need to be calculated based on recommended working concentrations for MicroFluere®.

Assay Procedure

When mixing antibody working solutions DO NOT vortex/mix them. Only pipette up and down. Vortex is okay for HRP/Substrate (Please see more detail in liquid handling tips section.)

1. Make a working solution of capture antibody; Dispense 20 μL in each well– ensure the liquid flows through the channels; Incubate 60 minutes and then drain the solution using the drainage device

2. Dispense 20 μL of blocking buffer in each well; Incubate 30 minutes and then drain the solution using the drainage device

After this step, the plate can be stored in cool and dry place for future use.

3. Make standards start with the highest concentration and serial dilutions; Dispense 20 μL in each well; Incubate 15 minutes and then drain the solution using the drainage device

In this step, samples can be loaded together with calibration standards.

4. Make a working solution of detection antibody; Dispense 20 μL in each well; Incubate 15 minutes and then drain the solution using the drainage device

5. Make a working solution of HRP; dispense 15 μL in each well; Incubate 5 minutes and then drain the solution using the drainage device

6. Wash with washing buffer (i.e., dispense 20 μL in each well and then drain the solution using the drainage device), repeat 2 times

7. Wash with PBS buffer (i.e., dispense 20 μL in each well and then drain the solution using the drainage device)

8. Make working solution of substrate (50:50:1 peroxide: enhancer: ADHP concentrate QuantaRed); Dispense 13 μL/well and incubate covered 5-15 minutes. Do not use stop solution. Do not drain the substrate. The plate is ready to read with a fluorescence microplate reader.

9. Read with a Fluorescence microplate reader (~550 nm excitation and 605 nm emission wavelengths)

Drainage device note: Insert a new absorbent pad and closes the lid. Wait for ~30 seconds before pressing the pump three to five times. Wait for ~20 seconds, then remove the plate. (Please see more detail in the Drainage Device video.)

Sample preparations note: Sample can be diluted in 1x of Reagent Diluent Concentrate 2 (1% BSA) with desired dilution.