PerkinElmer Life and Analytical Sciences
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Technical Data Sheet

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Wheat Germ Agglutinin FlashPlate® PLUS
96-well Wheat Germ Agglutinin Coated FlashPlate Microplates
SMP105, SMP105A

Product Description

This product is a 96-well FlashPlate coated with Wheat Germ Agglutinin (WGA). The interior of each FlashPlate well is permanently coated with a thin layer of polystyrene-based scintillant. The FlashPlate is then coated with proprietary proteins followed by Wheat Germ Agglutinin. This provides a platform for homogeneous, high-throughput receptor-ligand binding assays.

Wheat Germ Agglutinin (WGA) is a dimeric protein with a molecular weight of 36 kDa. It is a plant lectin which can bind carbohydrate residues on glycosylated proteins. The simplest sugar structure recognized by WGA is N-acetylglucosamine (NAG). WGA-coated FlashPlates capture receptors having these carbohydrate moieties.

Product Application

Wheat Germ Agglutinin FlashPlate PLUS microplates are suitable for the capture of membrane receptors for use in high-throughput receptor binding assays using ^{125}I-labeled ligands.

Materials Included

WGA FlashPlate PLUS, 5 Plate Pack
Catalog #: SMP105
96-well microplates - 5
TopSeal™ Microplate Covers - 10
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WGA FlashPlate PLUS, 20 Plate Pack
Catalog #: SMP105A
96-well microplates - 20
TopSeal Microplate Covers - 40
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Storage and Stability

Store plates at 2-8°C with desiccant and protect from direct sunlight. Plate is stable through the expiration date.

Equipment Required

FlashPlate microplates are designed for use with the PerkinElmer TopCount® Microplate Scintillation and Luminescence Counter. Software is pre-set for the various isotopes that may be used with FlashPlates. Refer to the instrument manual for detailed instructions on the appropriate settings to use.

The PerkinElmer MicroBeta® Trilux may also be used to count FlashPlate microplates. The bottom set of photomultiplier tubes must be shut off and the system programmed for the appropriate plate holder cassette.

Detailed information on the use of both instruments is available. Be sure that counting instrument is set for the appropriate isotope and counting conditions.

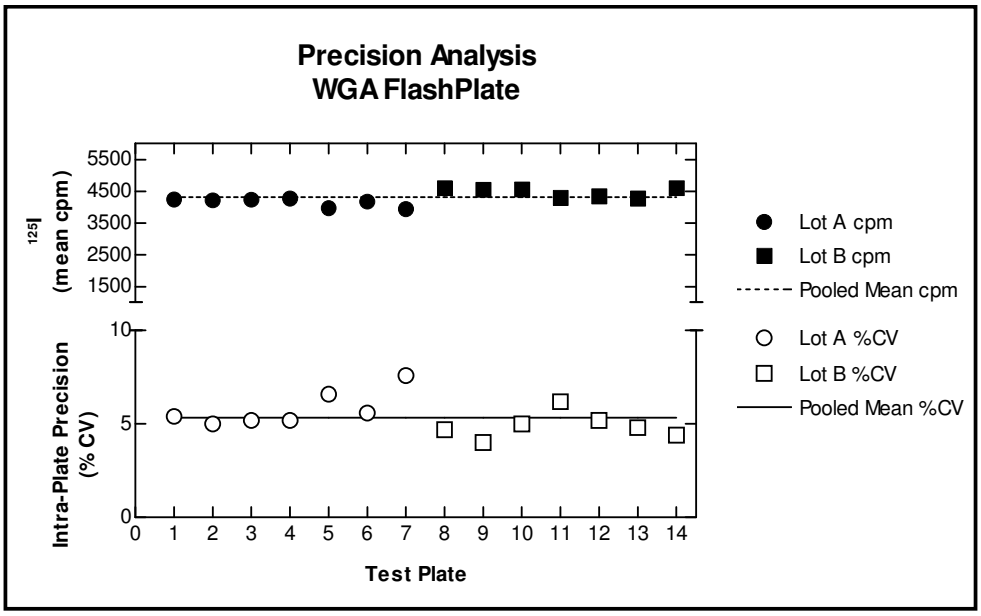
Coating Volume

The WGA FlashPlate PLUS has been coated with a volume of 200 µL/well.

Performance Characteristics

Intra-Plate Precision

The intra-plate precision of the WGA FlashPlate PLUS was tested by assaying an IC_{50} concentration of [^{125}I]-glycophorin[1] on 40 wells in 7 plates each of two different plate lots. The intra-plate % CV and mean cpm value for each test plate is reported below. Inter-plate CV's in this experiment were 5.8% and 4.9% for lots 1 and 2 respectively.



Reagent Tolerances

The DMSO and detergent conditions described below were tested in a glycophorin assay system. These reagents may still interfere with specific assay systems. Each researcher must determine if his/her assay system is sensitive to these reagents.

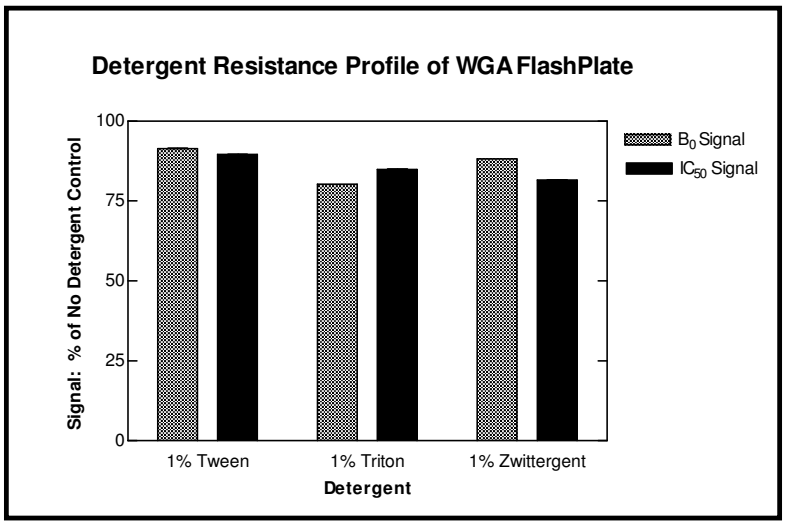
DMSO

DMSO concentrations up to 10% did not adversely affect the ability of WGA FlashPlates PLUS microplate to bind [^{125}I]glycophorin. The plate can tolerate at least overnight incubation with 10% DMSO in the assay buffer and short exposures of 50% DMSO. The direct addition of 100% DMSO to the plate will cause a significant loss in signal. This signal loss correlates with the volume of 100% DMSO added.

[1] Glycophorin is an erythrocyte membrane glycoprotein used to assess the precision of the WGA coating process on the FlashPlate. WGA is known to agglutinate erythrocytes and has been used to purify glycophorin.

Detergents

Detergent sensitivity was tested by adding 1% final concentration of detergent or no detergent to assay buffer and running an [^{125}I]-glycophorin binding assay. N = 24 for each determination. B_0 and IC_{50} signal is plotted as a % of no detergent control. This data demonstrates that the WGA FlashPlate is compatible with up to 1% concentrations of Triton X-100, Tween-20 or Zwittergent in the assay buffer.



Receptor-Ligand Binding Assay Application Data

Figure 1

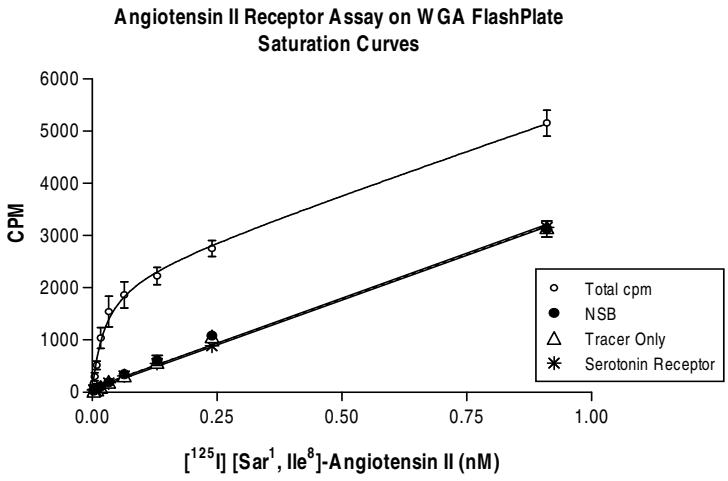
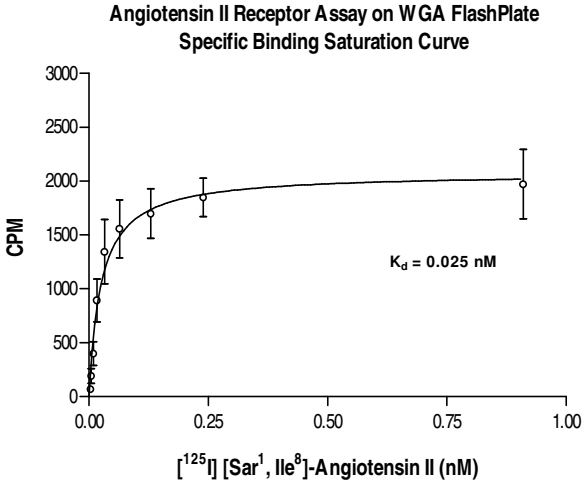


Figure 2



Angiotensin II Receptor Assay on WGA FlashPlate

A saturation curve on WGA FlashPlates (Figure 1) was determined for Cloned Human Angiotensin II receptor subtype 2 from HeLa Cells (PerkinElmer Cat.# CRM-069, B_{max} = 1.5 pmole/mg membrane protein). Angiotensin receptor was added at 2 μg/well in the presence of increasing amounts of [^{125}I][Sar^1, Ile^8]-Angiotensin II tracer (PerkinElmer Cat.# NEX248). The reported K_d for [Sar^1, Ile^8]-Angiotensin II and this receptor is 0.16 nM.

Non-specific binding (NSB) was determined in the presence of 0.2 μM cold [Sar[1], Ile[8]]-Angiotensin II. "Tracer Only" represents cpm for wells without receptor. "SeratoninR" represents a negative control of an irrelevant receptor added at the same concentration (2 μg/well) and made in the same cell line (HeLa) as the Angiotensin receptor. The three control curves are superimposable. Error bars represent the 95% confidence interval around the mean cpm (± 1.96 x SD). N=3 for each point. Figure 2 shows data from the same experiment corrected for NSB. The K_d determined from this experiment is 0.025nM, close to the reported literature value of 0.16 nM.

Assay Development Recommendations: Receptor-Ligand Binding Assays on WGA FlashPlate

Receptor – Ligand Selection

- Receptor-ligand combinations that work best on WGA FlashPlates generally meet the following criteria:
  - Receptor Expression: $B_{max} \approx 1$pM/mg protein
  - ^{125}I Ligand
  - Ligand Affinity: ≈1nM
- It is recommended that the optimal concentration of membrane receptor for an assay be determined by titration. Generally, 1 to 10 μg/well of membrane preparation will give optimal conditions. The optimal concentration is dependent on the receptor expression (B_{max}), ligand specific activity and non-specific binding. A high expression level or purity of the receptor can overcome some of the requirements listed above.

Buffers

- Detergents, proteins or other additives may help reduce non-specific binding. However, these agents may also prevent ligand-receptor binding or prevent receptor preparations from binding to the plate. Specific systems have different sensitivities and which must be determined by the investigator. Detergents are generally not recommended in the assay buffer formulations for most receptors and should not be used unless required.
- If a given receptor-ligand system has been characterized on a assay different technology, such as filtration, the buffer system for that technology provides a useful starting point for buffer optimization.

Assay Procedure

- WGA FlashPlates can be used either by simultaneously incubating receptors membrane and ligands or by pre-coating plates with receptor membranes prior to ligand addition. Simultaneous incubation of receptor with ligand is often preferable because the number of assay steps is minimized.
- Incubation time for receptor/ligand simultaneous addition is generally a minimum of 3 hours at ambient temperature to a maximum of 24 hours or more. This varies depending upon the properties of the receptor-ligand interaction and the desired performance criteria. Endogenous proteases in the membrane preparation may limit extended incubations unless appropriate precautions are used (e.g., protease inhibitors or incubation at 4°C).
- Assay kinetics should be examined in order to determine when the assay reaches equilibrium. The homogeneous assay format of the WGA FlashPlate allows you to take multiple readings of the

same plate over time. Assay kinetics can thus be determined by repeated readings of a receptor assay (without aspiration) at time intervals until equilibration has been reached. Shaking the plate during incubation may help drive kinetics to equilibrium sooner. The kinetics should be determined at a low ligand concentration (e.g., 10 to 20% of the K_d).

- It is not recommended that WGA plates pre-coated with membranes/receptors be stored for more than a few days unless the user has established a method of stabilizing the receptor onto the plate. If storing coated plates, seal the plates and keep them at 4°C.
- Aspiration or aspiration and wash steps at the end of the assay can help reduce non-specific binding and improve signal-to-noise ratios.
- Recommended total assay volume is 100 to 200 µL per well.

Receptor, Ligand Concentrations

- If the K_d of a receptor is known, the fraction of receptor binding sites occupied at a given ligand concentration is given by the following equation:

  fraction of receptor sites occupied by ligand = [ligand] / ([ligand] + K_d)

  This equation can be used to estimate the range of ligand concentrations needed for a saturation assay. For example, if the ligand concentration used is 9 x K_d then 90% of the sites will be occupied. The following table may be useful for estimating the concentration of ligand needed for a given receptor occupancy.

% Receptor Occupancy	[Ligand] as multiple of K_d
95%	19.00
90%	9.00
85%	5.67
80%	4.00
75%	3.00
50%	1.00
25%	0.33

Curve fitting will be more accurate if the ligand concentration includes points near receptor saturation as well as points spanning a broad enough range to draw a representative curve.