

Receptor Binding Assays on WGA FlashPlate[®]

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Introduction

Wheat Germ Agglutinin (WGA) is a plant lectin that binds carbohydrate residues on glycosylated proteins, providing a means for capturing cloned receptor membranes on the FlashPlate® surface. WGA FlashPlates are white, opaque, 96- and 384-well scintillant coated microplates which are coated with WGA. This platform enables receptor-ligand binding assays that are homogeneous, easily automated and fully compatible with miniaturized HTS. WGA FlashPlates are very suitable for high throughput screening and provide reproducible and dependable results.

Assays for receptor/ligand pairs, such as chemokines, are often difficult to optimize due to non-specific binding interactions of the ligand with proteins other than the specific receptor. FlashPlate technology provides the flexibility to optimize such assays. WGA FlashPlates provide the options of non-specific binding blockers, aspiration steps, and wash steps, when appropriate, to increase signal-to-noise. The flexibility provided by the WGA FlashPlates allows formatting of assays which otherwise may not be suitable for HTS.

New applications have been developed on WGA FlashPlates to measure the binding interactions between several receptor/ligand binding pairs. Data is presented at both 96- and 384-well densities for a variety of G protein-coupled receptor binding assays using ¹²⁵I-labeled ligands. Pharmacology profiles are compared to a standard ¹²⁵I filter assay.

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Methods, Results, and Discussion

Materials

- WGA FlashPlate (96-well, NEN, Cat. # SMP105)
- WGA FlashPlate (384-well, NEN, Cat. # SMP411)
- Polyethylenimine (PEI) (Aldrich, Cat. # 40,870-0)

- [¹²⁵I]Nociceptin (NEN, Cat. # NEX338)
- ORL-1 Receptor (RBI, Cat. # RB-HORL)
- Orphanin FQ (Sigma, Cat. # 04011)

- [¹²⁵I]IL-8 (NEN, Cat. # NEX277)
- CXCR1 Receptor (NEN, Cat. # CRM076)
- CXCR2 Receptor (NEN, Cat. # CRM056)

- [¹²⁵I]MIP-1α (NEN, Cat. # NEX298)
- CCR1 Receptor (NEN, Cat. # CRM057)

- [¹²⁵I]I-309 (NEN, Cat. # NEX364)
- CCR8 Receptor (Euroscreen, Cat. # ES-136-M)

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Assay Protocols

All FlashPlate assays were run in a homogeneous format. In some assays, aspiration and wash steps were performed to increase signal-to-noise ratios. Receptor, ^{125}I ligand, and cold compounds were added to the FlashPlate and incubated overnight. FlashPlates were read on a TopCount[®] Microplate Counter.

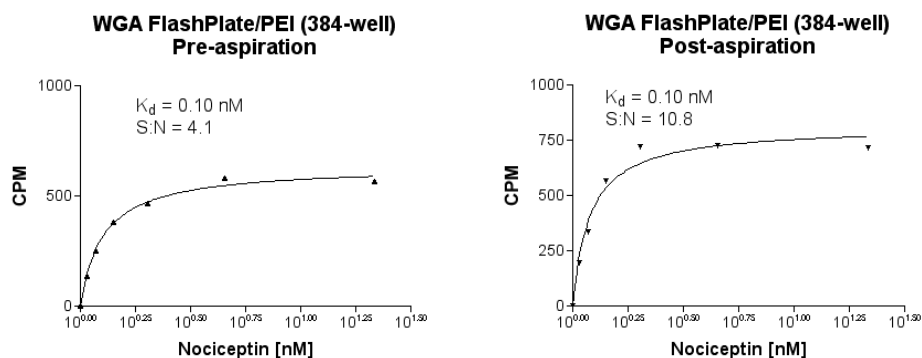
Filter assays were performed according to typical standard methods; all reagents were added simultaneously and incubations varied from 1-3 hours depending on the assay. In some assays, the filter paper was first treated with polyethylenimine (PEI).

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Nociceptin/ORL-1 Receptor

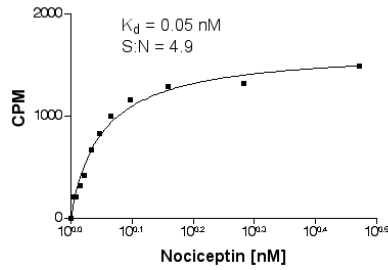
In order to develop a robust and valid assay, studies were done to determine optimal assay conditions in both 96-well and 384-well formats.

Initial studies were done using WGA FlashPlates without a special blocking step. The signal:noise ratio was too low, resulting in an assay that was not feasible. The assay was then evaluated with the addition of a 0.1% PEI blocking step to the WGA FlashPlate. In addition to the PEI blocking step, studies were done to determine if aspirating and washing could enhance the signal:noise.

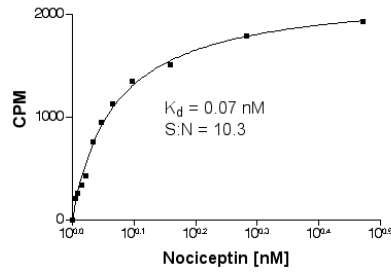


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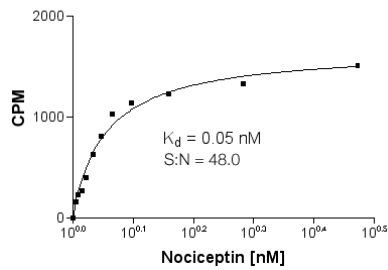
**WGA FlashPlate/PEI (96-well)
Pre-aspiration**



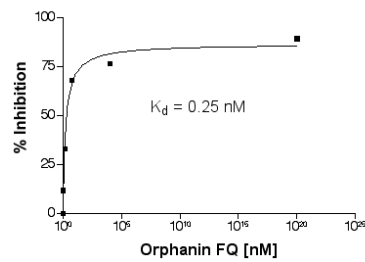
**WGA FlashPlate/PEI (96-well)
Post-aspiration**



**WGA FlashPlate/PEI (96-well)
Post-aspiration and 1 Wash**

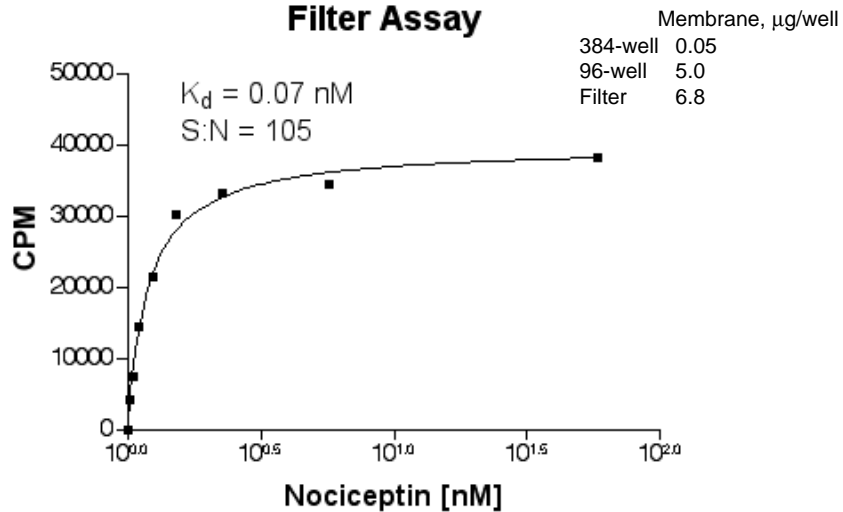


**WGA FlashPlate/PEI (96-well)
Competition Assay
Post-aspiration and 1 Wash**



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Filter Assay



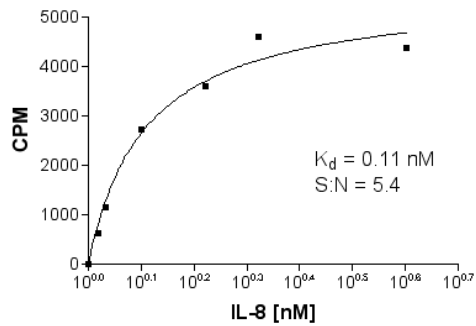
As shown above, valid results were obtained with all conditions. FlashPlate technology resulted in a more robust assay with a valid K_d and improved S:N by adding an optional aspiration and wash step.

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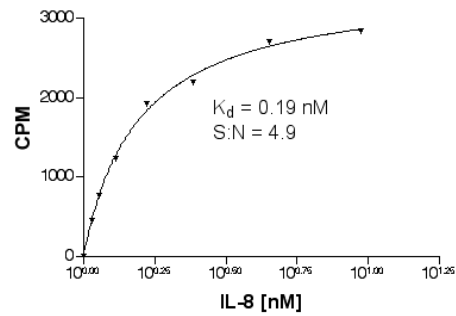
IL-8/CXCR1 Receptor

As with the ORL-1 receptor, the K_d and signal:noise did not result in a feasible assay without using a PEI blocking step. Results with a PEI blocking step are shown in both the 96- and 384-well formats.

WGA FlashPlate/PEI (96-well)

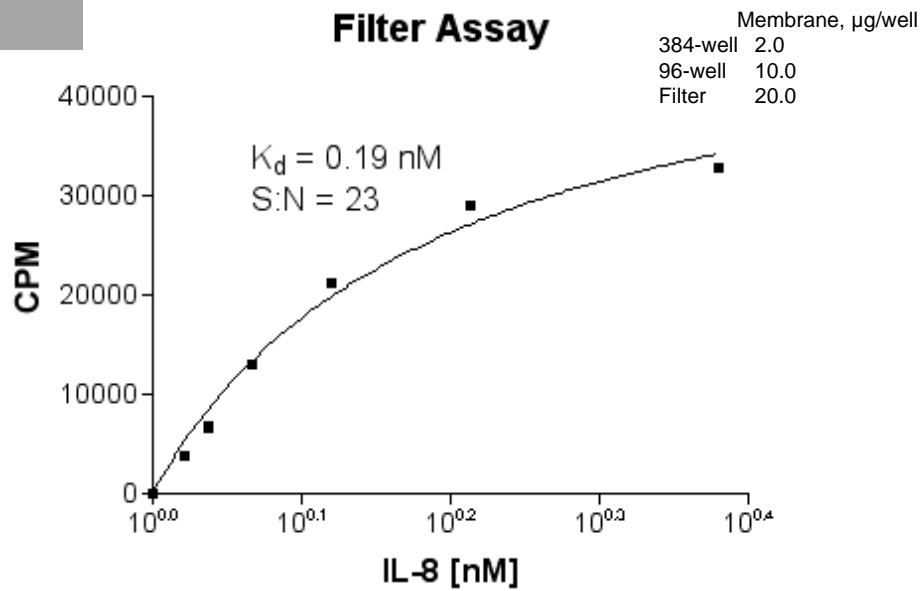


WGA FlashPlate/PEI (384-well)



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Filter Assay



As shown above, results show similar K_d values using the WGA FlashPlate as compared to a filter assay.

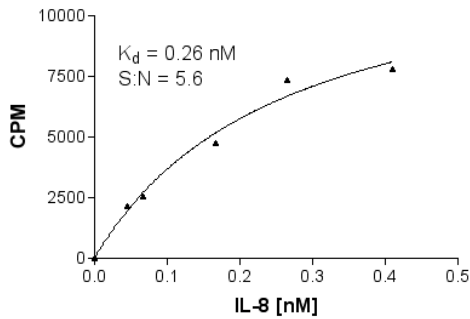
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IL-8/CXCR2 Receptor

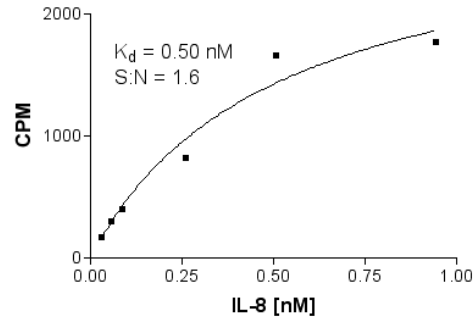
Data below demonstrates a 96-well FlashPlate assay vs. a filtration assay for [¹²⁵I]IL-8 and CXCR2 receptor. Both the FlashPlate and the filter paper were treated with PEI.

	Membrane, µg/well
96-well	5.0
Filter	9.0

WGA FlashPlate/PEI (96-well)



Filter Assay



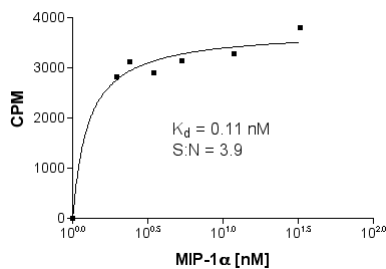
Results show similar K_d values using the WGA FlashPlate as compared to a filtration assay. The filter assay was not run under optimized conditions.

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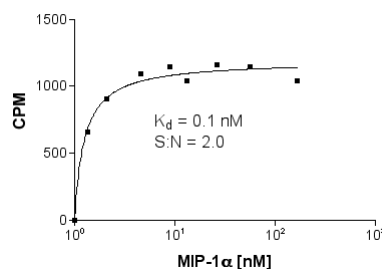
MIP-1 α /CCR1 Receptor

Preliminary studies were performed with MIP-1 α and CCR1 receptor to show feasibility in both 96- and 384-well formats vs. a filter assay.

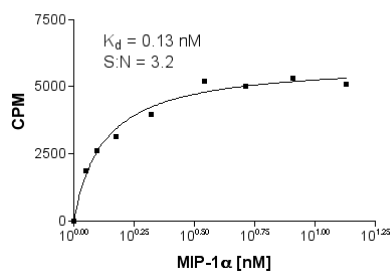
WGA FlashPlate (96-well)



WGA FlashPlate (384-well)



Filter Assay



Results show similar K_d values and S:N using the WGA FlashPlate as compared to a filter assay. These preliminary graphs show feasibility, but results are not optimal. To increase the S:N, aspiration and washing studies will be evaluated.

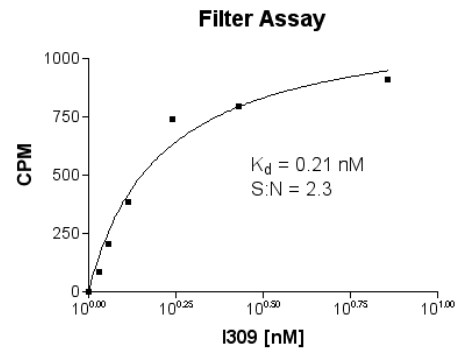
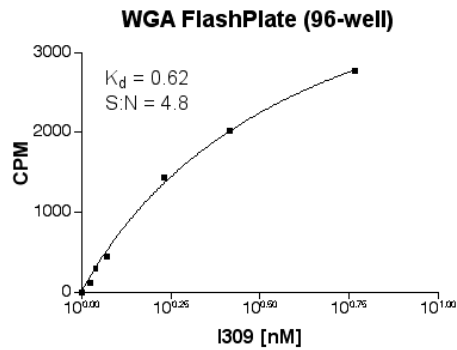
	Membrane, µg/well
384-well	4.0
96-well	20.0
Filter	37.0

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I309/CCR8 Receptor

Preliminary studies were done with I309 and CCR8 receptor to show feasibility in 96-well format vs. a filter assay. PEI blocking was not attempted.

	Membrane, $\mu\text{g}/\text{well}$
96-well	1.0
Filter	2.5



Conclusion

Receptor ligand binding was successfully demonstrated in a homogeneous format on WGA FlashPlates. This flexible technology is effective for formatting assays on difficult receptor classes, such as chemokines. When working with sticky ligands, the option to aspirate and wash to help increase signal:noise yields a more robust assay, suitable for HTS at both the 96- and 384-well density. Pharmacology of the FlashPlate assays is consistent with results obtained from standard filtration assays. In contrast to filtration, the FlashPlate protocol is fully homogeneous and easily miniaturized to 384-well density.

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