

PROSTANOID RECEPTOR ARRAY READY-TO-ASSAY PLATES FROZEN PREPLATED MEMBRANE PREPARATION (96-well)

CATALOG NUMBER:	HTS900PA	QUANTITY:	One 96-well plate containing EP3, EP4, DP, CRTH2 and TP membrane preparations
LOT NUMBER:		PRESENTATION:	GPCR membranes preparations are supplied at 10 µg in 25 µL (1 unit) per well, in each well of the plate. The membranes are formulated in 50 mM Tris pH 7.4, 10% glycerol and 1% BSA with no preservatives.

BACKGROUND: Traditional GPCR radioligand binding assays performed by filtration require assembly of the binding reaction in an assay plate, followed by transfer of the reaction to a PEI-coated glass fiber filter plate for filtration. Millipore currently offers MultiScreen_{HTS} FB and FC plates with a deep well that permits the binding reaction to be performed in the filter plate, thereby eliminating the need for separate filtration and assay plates. Millipore has now developed Ready-To-Assay Plates, which contain ChemiScreen GPCR membrane preparations pre-frozen on a MultiScreen_{HTS} FC plate at an optimized amount of 1 unit per well.

Prostanoids are a series of arachidonic acid metabolites produced by the action of cyclooxygenase and further modified by isomerases and synthases. Cells rapidly secrete prostanoids after synthesis, whereupon the prostanoids bind to a family of 8 GPCRs to exert their biological effects (Narumiya and FitzGerald, 2001; Hata and Breyer, 2004). Millipore's Prostanoid Receptor Array contains membrane preparations for two prostaglandin E2 receptors (EP3 and EP4), two prostaglandin D2 receptors (DP and CRTH2), and the thromboxane A2 receptor (TP). The prostaglandin PGE₂ causes pain, vasodilation, immunosuppression of T cells, bone resorption and promotion of carcinogenesis. Four related GPCRs, EP₁, EP₂, EP₃ and EP₄, each bind to PGE₂, but the different G protein coupling status of each receptor leads to distinct biological effects. The prostaglandin PGD₂ is produced by mast cells upon activation by allergens, and is present at high levels in allergic diseases. PGD₂ binds to two receptors, DP and CRTH2. Thromboxane A₂ receptor (TP) is widely distributed among different organ systems and have been localized on both cell membranes and intracellular structures. TP receptors belong to G protein-coupled receptor family (Hirata *et al.*, 1991). Activation of TP receptors induces platelet aggregation and vascular and respiratory smooth muscle constriction and cell growth. Chemicon's Prostanoid Receptor membrane preparations are crude membrane preparations made from our proprietary stable recombinant cell lines to ensure high-level of GPCR surface expression; thus, they are ideal HTS tools for screening of agonists and antagonists of prostanoid receptors.

The Recombinant Human Prostanoid Receptor Array (96-well) exhibits comparable signal-to-background ratios, and K_d and B_{max} values to those observed with a traditional harvest plate method.

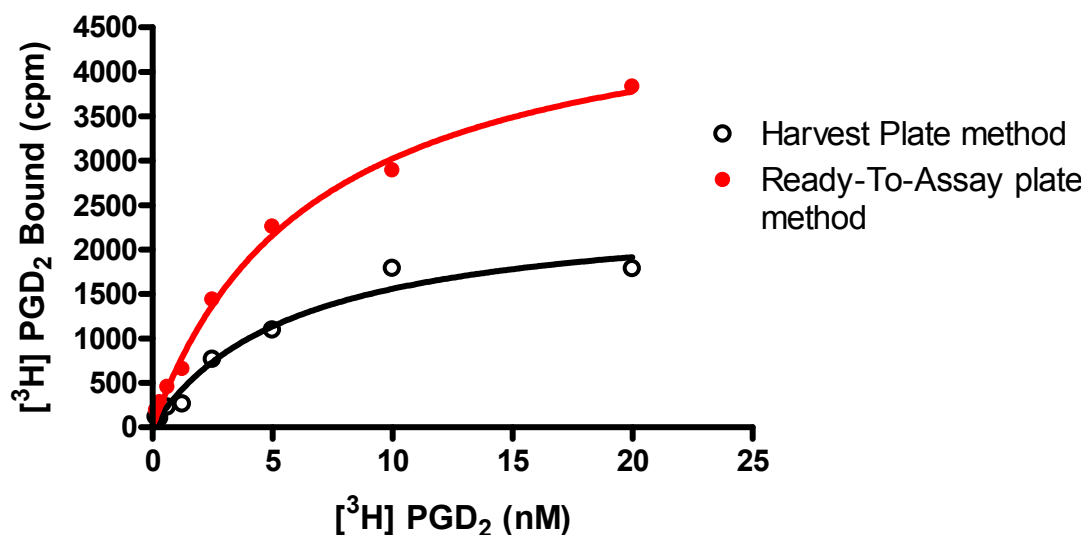
APPLICATIONS: Radioligand binding assay

Plate layout

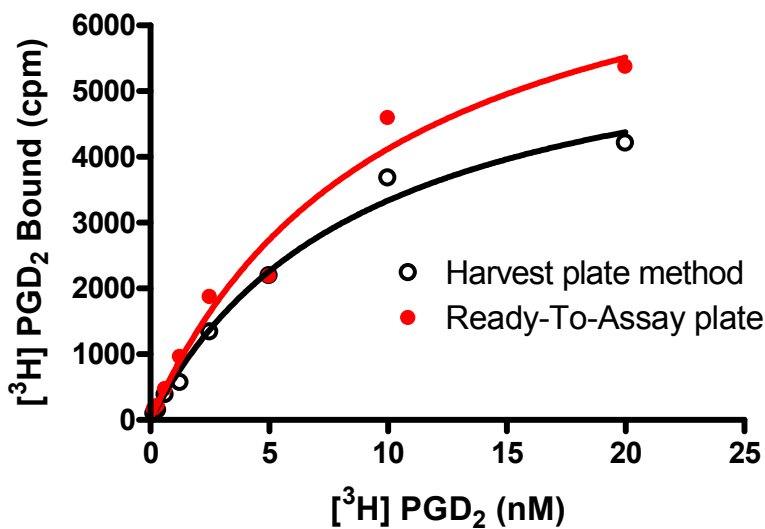
	1	2	3	4	5	6	7	8	9	10	11	12
A	DP	DP	CRTH2	CRTH2	TP	TP	EP3	EP3	EP4	EP4		
B	DP	DP	CRTH2	CRTH2	TP	TP	EP3	EP3	EP4	EP4		
C	DP	DP	CRTH2	CRTH2	TP	TP	EP3	EP3	EP4	EP4		
D	DP	DP	CRTH2	CRTH2	TP	TP	EP3	EP3	EP4	EP4		
E	DP	DP	CRTH2	CRTH2	TP	TP	EP3	EP3	EP4	EP4		
F	DP	DP	CRTH2	CRTH2	TP	TP	EP3	EP3	EP4	EP4		
G	DP	DP	CRTH2	CRTH2	TP	TP	EP3	EP3	EP4	EP4		
H	DP	DP	CRTH2	CRTH2	TP	TP	EP3	EP3	EP4	EP4		

Note: columns 11 and 12 are pretreated but contain no membrane preparations. These wells may be used for spotting with radioligand solution to determine total counts applied, or may be used to assay the user's membrane preparations in parallel with the preloaded membrane preparations.

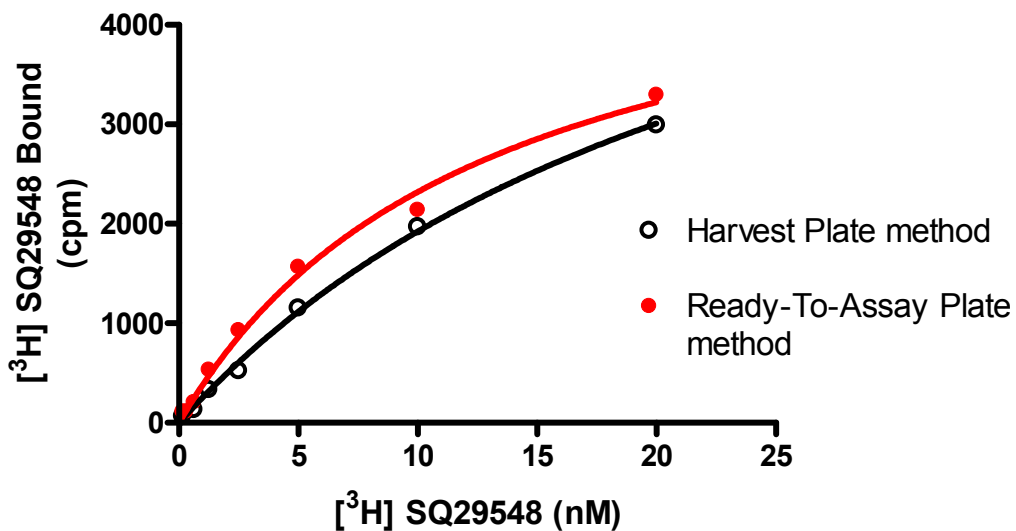
DP



CRTH2



TP



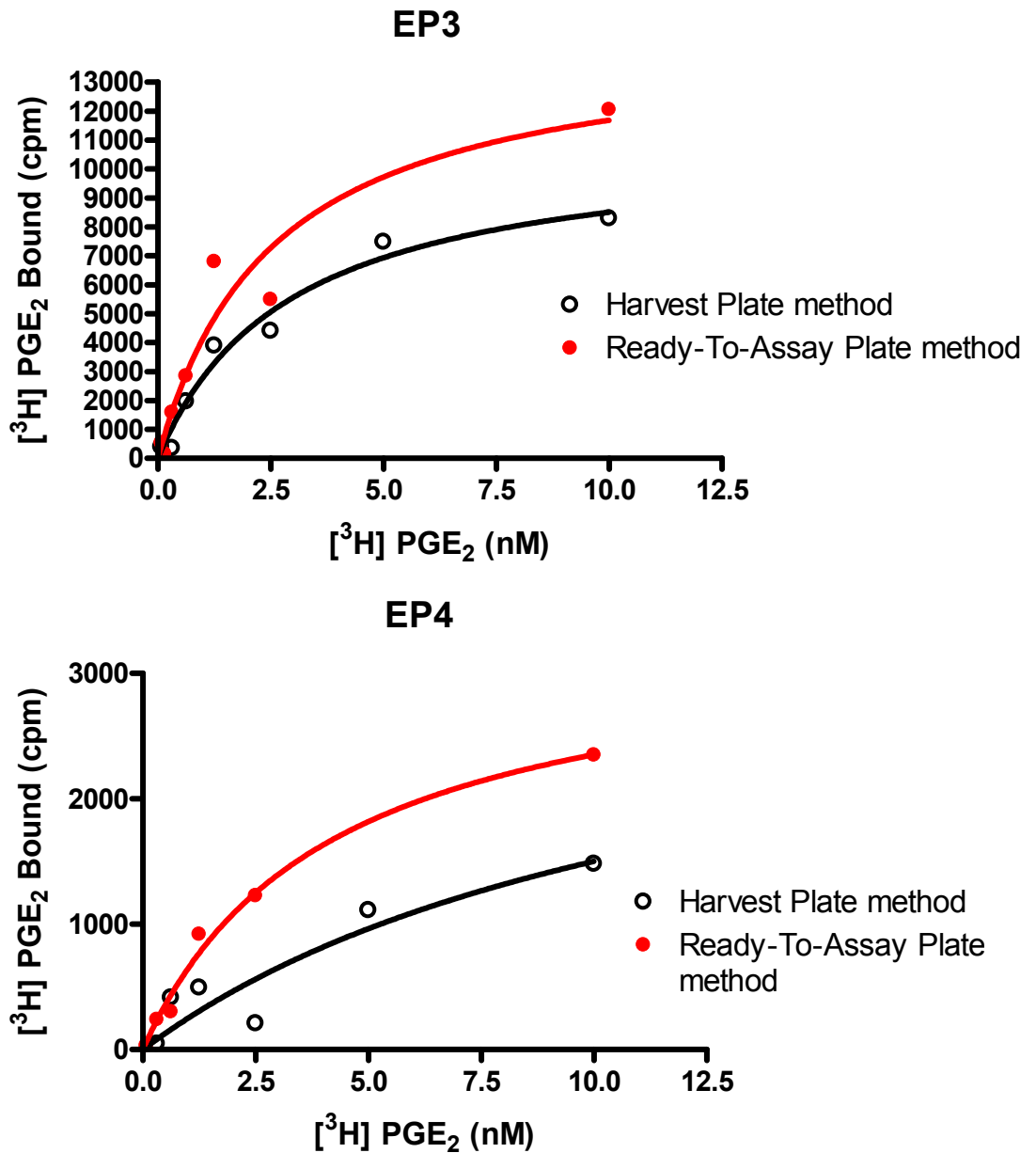


Figure 1. Comparison of harvest plate and Ready-To-Assay plate methods in saturation binding with Prostanoid Receptors.

Table 1. Kd and Bmax values for prostanoid receptors obtained in saturation binding with harvest plate and Ready-To-Assay plate methods

	Kd (nM) with Ready-To- Assay Plate	Kd (nM) with harvest plate method	Bmax with Ready-To- Assay plate	Bmax with harvest plate method
DP	6.6	5.9	15.2	10.3
CRTH2	10.2	9.1	25.1	26.3
TP	12.7	25.9	55.4	77.1
EP3	2.5	3.2	28.8	33.5
EP4	4.2	12.6	6.5	10.1

Frozen Preplated Membrane Method: Performed as described in "RECOMMENDED ASSAY PROTOCOL FOR COMPETITION BINDING" below.

Harvest Plate method: Membrane preparations (Chemicon cat. #) were thawed rapidly and chilled on ice. Binding reactions consisting of unlabeled and labeled ligands at the concentrations indicated, 10 ug/well membrane preparation in binding buffer was assembled in an assay plate (Corning). The reaction was incubated for 2 h at room temperature, during which time a MultiScreen Harvest Plate (Millipore cat. # MAHF C1H) was incubated for 15 min with 0.3% PEI and washed with 50mM HEPES, pH 7.4, 0.5% BSA. Binding reaction was transferred to the filter plate, and washed 3 times (1 mL per well per wash) with Wash Buffer. The plate was dried and counted in top read mode.

Table 2. References for prostanoid receptor cDNAs used to prepare membrane preparations in the Prostanoid Receptor Array

	Gene name	Accession number
DP	PTGDR	NM_000953
CRTH2	GPR44	AB008535
TP	TBXA2R	NM_001060
EP3	PTGER3 splice variant 6	NM_198716
EP4	PTGER4	NM_000958

HOST CELLS: Chem-1, an adherent mammalian cell line without endogenous prostanoid receptor expression.

RECOMMENDED ASSAY PROTOCOL FOR COMPETITION BINDING:

1. Thaw the plate at room temperature with the lid in place.
2. Add to the thawed membranes in the plate: radiolabeled ligand (see table 1 below for specific ligand and concentration), test compound and binding buffer for a final volume of 100 μ L (the volume of preplated GPCR membrane is 25 μ L).
3. Cover the plate with the lid and incubate for 1-2 h at room temperature.
4. Stop reaction by addition of 100 μ L/well binding buffer.
5. Filter with MultiScreen_{HTS} Vacuum Manifold.
6. Wash 3x with Wash Buffer, 200 μ L/well/wash.
7. Remove the underdrain from the bottom of the plate.
8. Dry the filter plate.
9. Seal the bottom with clear tape
10. Add 50 μ L/well scintillation cocktail.
11. Seal the top of plate with clear tape, and count in Microbeta scintillation counter on coincidence mode

Binding Buffer: 50 mM Hepes pH 7.4, 5 mM MgCl₂, 1 mM CaCl₂, 0.2% BSA, filtered and stored at 4°C

Wash Buffer: 50 mM Hepes, pH 7.4, 500mM NaCl , 0.1% BSA filtered and stored at 4°C.

Table 3. Radioligands for binding assay with Prostanoid Receptor Array

	radioligand	Recommended concentration for competition	Vendor/part number
DP	[³ H]-Prostaglandin D ₂	5 nM	Perkin Elmer NET616; Amersham TRK734
CRTH2	[³ H]-Prostaglandin D ₂	10 nM	
TP	[³ H]-SQ 29548	10 nM	Perkin Elmer NET936
EP3	[³ H]-Prostaglandin E ₂	1.5 nM	Perkin Elmer NET428: Amersham TRK431
EP4	[³ H]-Prostaglandin E ₂	3 nM	

STORAGE/HANDLING: Maintain frozen at -70°C for up to 2 years. Do not freeze and thaw.

REFERENCES: Hata AN and Breyer RM (2004) Pharmacology and signaling of prostaglandin receptors: multiple roles in inflammation and immune modulation. *Pharmacol. Ther.* 103: 147-166.
Narumiya S and FitzGerald GA (2001) Genetic and pharmacological analysis of prostanoid receptor function. *J. Clin. Invest.* 108: 25-30.

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