Validation & Assay Performance Summary

GeneBLAzer® RXR beta DA Cells & Assay Kit

GeneBLAzer® RXR beta-UAS-*bla* HEK 293T Cells

Cat. no. K1405, K1694

Target Description

The Retinoid X receptor-beta (RXR beta) is a nuclear hormone receptor and can function as a ligand inducible transcription factor capable of acting as a co-repressor and/or co-activator for gene expression. Nuclear receptors contain a series of conserved domains or regions. These domains/regions include a variable NH_2-domain (A/B region), a conserved DNA-binding domain (DBD or region C), a linker region (region D), a ligand binding domain (LBD or region E), and in some receptors a variable COOH-terminal (region F) (1). RXR beta belongs to the family of retinoid x receptors, one of two retinoic receptor families (retinoic acid receptors and retinoid x receptors). RXR beta is one of three members of the RXR family which consists of RXR alpha, RXR beta, and RXR gamma (11). The A/B and D regions of RXR beta are involved in dictating the cell dependent transcriptional response (6).

RXR receptors are able to form both homo- and heterodimers (3). RXR receptors have been reported to form heterodimers with TRs (thyroid hormone receptors), RXRs retinoic acid receptors), VDR (vitamin D receptor), PPARs (peroxisome proliferator activated receptors), LXRs (liver x receptors), and FXR (farnesoid x receptor). These heterodimers can be classified as permissive and nonpermissive heterodimers (3). Addition of an RXR agonist, such as 9-cis-retinoic acid, can result in transcriptional activity of permissive heterodimers while activation of nonpermissive heterodimers occurs independent of the RXR agonist (3). RXR agonist LG100268 activates the transcriptional response RXR:PPARγ and RXR:LXR heterodimers alone and synergistically with PPARγ and LXR agonists, but does not activate RXR:RXR and RXR:TR heterodimers whose activity is dependent upon RXR and TR agonists (10).

RXR beta is widely expressed in almost every tissue. The generation of RXR beta null mice resulted in approximately 50% of the mice to die before birth and those that survived appear normal, except for male sterility (reviewed in 11).

The endogenous ligands for RXR beta include 9-cis-retinoic acid (4,6), phytanic acid (12), and docosahexaenoic acid (7). Synthetic agonists for RXR beta have been termed rexinoids and includes LG100268 (10).

Cell Line Description

GeneBLAzer® RXR beta DA (Division Arrested) cells and RXR beta-UAS-*bla* HEK 293T cells contain the ligand-binding domain (LBD) of the human Retinoid X Receptor beta (RXR beta) fused to the DNA-binding domain of GAL4 stably integrated in the GeneBLAzer® UAS-*bla* HEK 293T cell line. GeneBLAzer® UAS-*bla* HEK 293T cells stably express a beta-lactamase reporter gene under the transcriptional control of an upstream activator sequence (UAS). When an agonist binds to the LBD of the GAL4 (DBD)-RXR beta (LBD) fusion protein, the protein binds to the UAS, resulting in expression of beta-lactamase.

DA cells are irreversibly division arrested using a low-dose treatment of Mitomycin-C, and have no apparent toxicity or change in cellular signal transduction. Both RXR beta DA cells and RXR beta-UAS-*bla* HEK 293T cells are functionally validated for Z' and EC_{50} concentrations of *9-cis* retinoic acid (9-cis-RA) (Figure 1). In addition, RXR beta-UAS-*bla* HEK 293T cells have been tested for assay performance under variable conditions, including DMSO concentration, cell number, and stimulation time.

Validation Summary

Performance of this assay was validated under optimized conditions in 384-well format using LiveBLAzer™-FRET B/G Substrate.

1. **Primary agonist dose response under optimized conditions (n=3)**

	DA	Dividing
9-cis retinoic acid EC_{50}	4.8 nM	12 nM
Z'-Factor (EC_{100})	0.79	0.74

Response Ratio = 4-5
Optimum cell no. = 10K cells/well
Optimum [DMSO] = up to 1%
Stimulation Time = 16 hours
Max. [Stimulation] = 10 µM

2. **Alternate agonist dose response**

All-trans-retinoic acid	EC_{50} = 22.6 nM
Phytanic acid	EC_{50} = 6.9 µM
Docosahexaenoic acid	EC_{50} = 10 µM

3. **Antagonist dose response**
   See antagonist dose response section

4. **Cell culture and maintenance**
   See Cell Culture and Maintenance Section and Table 1

Assay Testing Summary

5. **Assay performance with variable cell number**

6. **Assay performance with variable stimulation time**

7. **Assay performance with variable substrate loading time**

8. **Assay performance with variable DMSO concentration**

9. **Dose Response with additional nuclear receptor agonists**

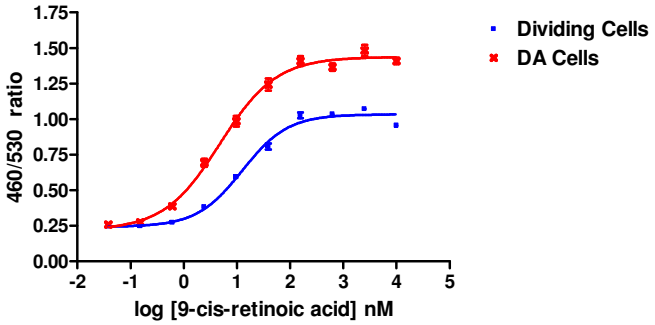
10. **Assay performance with variable assay plate types**

11. **Dose Response with additional nuclear receptor agonists in the presence of RXRα antagonist RO41-5253**

12. **Effect of pre-incubating cells on assay performance**

Primary Agonist Dose Response

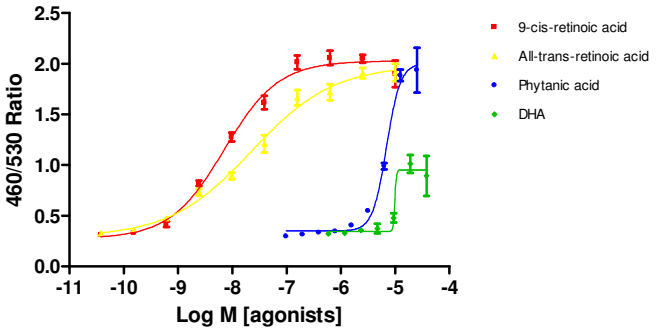
Figure 1 —9-cis-retinoic acid dose response under optimized conditions



Dividing RXR beta-UAS-*bla* HEK 293T cells were serum starved for 24 hrs prior to the assay, while division arrest cells were thawed and assayed directly. The day of the assay, cells were plated in a 384-well format (10,000 cells/well) and stimulated with 9-cis-retinoic acid (Biomol #GR101) over the indicated concentration range in the presence of 1.0% DMSO for 16 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate (1 µM final concentration of CCF4-AM) for 90 minutes. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader in bottom-read mode, and the background-corrected ratios were plotted against the indicated concentrations of 9-cis-retinoic acid (n= 16 for each data point).

Alternate Agonist Dose Response

Figure 2 —9-cis-retinoic acid, all-trans-retinoic acid, phytanic acid, and docosahexaenoic acid agonist dose Response



RXR beta-UAS-*bla* HEK 293T cells (10,000 cells/well) were serum starved for 24 hrs prior to the assay, and then plated the day of the assay in a 384-well format. Cells were stimulated with either 9-cis-retinoic acid (Biomol #GR101), all-trans-retinoic acid (Sigma #R2500), phytanic acid (Sigma cat# P4060) or docosahexaenoic acid (DHA) (Sigma #D2534) over the indicated concentration range in the presence of 1.0% DMSO for 16 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate (1 µM final concentration of CCF4-AM) for 90 minutes. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader in bottom-read mode, and the background-corrected ratios were plotted against the indicated concentrations of the agonists (n= 8 for each data point).

Antagonist Dose Response

There are currently no commercially available antagonists for RXR beta

Cell Culture and Maintenance

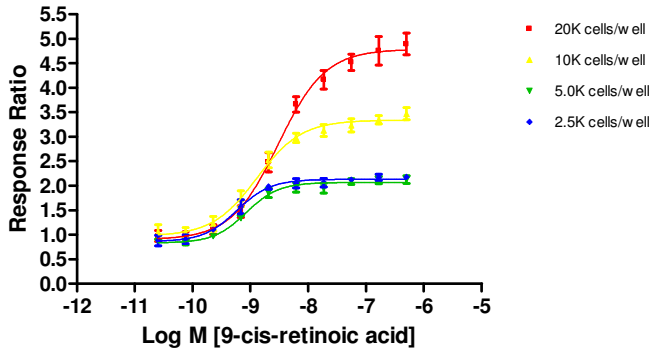
Cells should be maintained between 5 and 90% confluency in complete growth media and in a humidified incubator at 37ºC and 5% CO_2. Split cells at least twice a week. Do not allow cells to reach confluence.

Table 1 – Cell Culture and Maintenance

Component	Growth Medium (–)	Growth Medium (+)	Assay Medium	Freeze Medium
DMEM, w/ GlutaMAX™	90%	90%	—	—
Phenol Red free DMEM	—	—	98%	—
Dialyzed FBS **Do not substitute!**	10%	10%	—	—
Charcoal/Dextran FBS	—	—	2%	
NEAA	0.1 mM	0.1 mM	0.1 mM	—
HEPES (pH 7.3)	25 mM	25 mM	—	—
Hygromycin B	—	100 µg/mL	—	—
Zeocin™	—	100 µg/mL	—	—
Penicillin	100 U/mL	100 U/mL	100 U/mL	—
Streptomycin	100 µg/mL	100 µg/mL	100 µg/mL	—
Sodium Pyruvate	—	—	1 mM	
Recovery™ Cell Culture Freezing Medium	—	—	—	100%

Assay Performance with Variable Cell Number

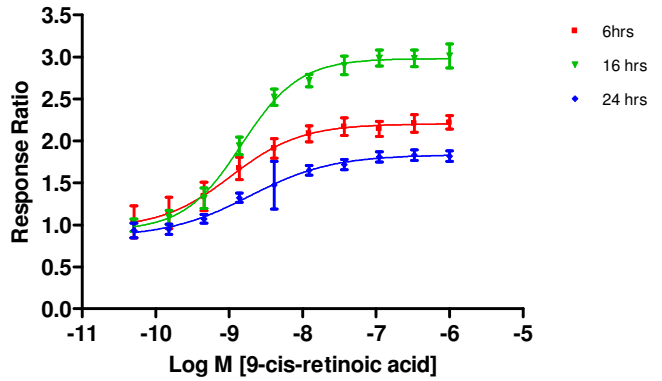
Figure 4— 9-cis-retinoic acid dose response with 2.5, 5.0, 10, and 20K cells/well



RXR beta-UAS-*bla* HEK 293T cells were plated at 2500, 5,000, 10,000 or 20,000 cells/well in a 384-well format and serum starved for 24 hours prior to the assay. The cells were then stimulated with a serial dilution of 9-cis-retinoic acid (Biomol #GR101) in the presence of 0.5% DMSO for 18 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate (1 µM final concentration of CCF4-AM) for 90 min. Fluorescence emission values at 460 nm and 530 nm for the various cell numbers were obtained using a standard fluorescence plate reader in bottom-read mode, and the background-corrected Response Ratios were plotted against the indicated concentrations of 9-cis-retinoic acid (n=8 for each data point).

Assay performance with Variable Stimulation Time

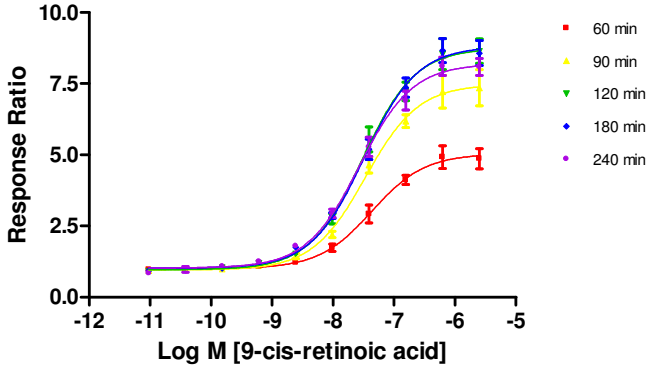
Figure 5 – 9-cis-retinoic acid dose response with 6, 16, and 24 hour stimulation times



RXR beta-UAS-*bla* HEK 293T cells (20,000 cells/well) were plated in a 384-well black-walled tissue culture assay plate and serum starved for 24 hours prior to the assay. The cells were then stimulated with a serial dilution of 9-cis-retinoic acid (Biomol #GR101) in the presence of 0.5% DMSO for 6, 16, and 24 hours. Cells were then loaded for 90 minutes with LiveBLAzer™-FRET B/G Substrate(1 µM final concentration of CCF4-AM). Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader in bottom-read mode, and the background-corrected Response Ratios were plotted against the concentrations of 9-cis-retinoic acid (n=8 for each data point).

Assay performance with Variable Substrate Loading Time

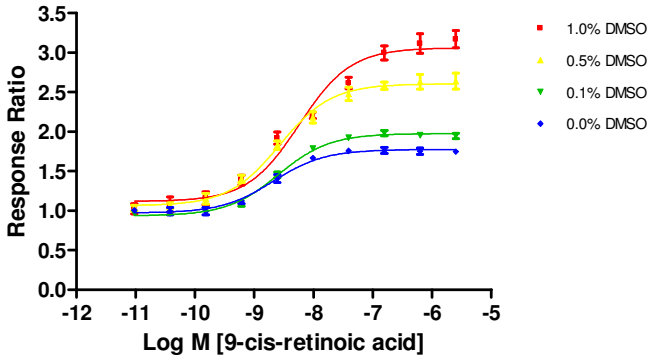
Figure 6 – 9-cis-retinoic acid dose response with 1, 1.5, 2, 3, and 4 hour loading times



RXR beta-UAS-*bla* HEK 293T cells were plated at 10,000 cells/well in a tissue culture treated 384-well plate format the day of the assay after a 24 hour starvation in assay media. Cells were stimulated with 9-cis-retinoic acid (Biomol #GR101) in the presence of 0.5% DMSO for 16 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate (1 µM final concentration of CCF4-AM) for either 1, 1.5, 2, 3, or 4 hours. Fluorescence emission values at 460 nm and 530 nm for the various loading times were obtained using a standard fluorescence plate reader in bottom-read mode, and the background-corrected Response Ratios were plotted against the indicated concentrations of 9-cis-retinoic acid (n=8 for each data point).

Assay Performance with variable DMSO concentration

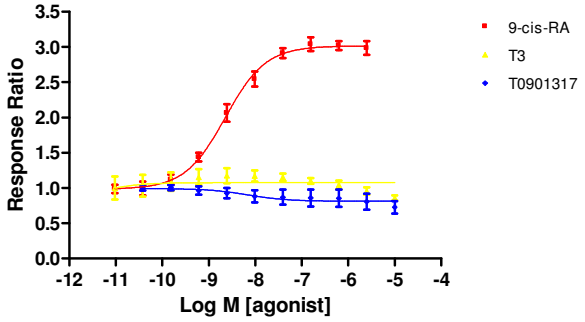
Figure 7 – 9-cis-retinoic acid dose response with 0, 0.1, 0.5 and 1% DMSO.



RXR beta-UAS-*bla* HEK 293T cells (20,000 cells/well) were plated the day of the assay in a 384-well black-walled tissue culture assay plate after a 24 hour starvation in assay media. 9-cis-retinoic acid (Biomol #GR101) was then added to the plate over the indicated concentration range. DMSO was added to the assay at concentrations from 0% to 1%. Cells were stimulated for 16 hrs with agonist and loaded for 90 minutes with LiveBLAzer™-FRET B/G Substrate (1 µM final concentration of CCF4-AM). Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader in bottom-read mode, and the background-corrected Response Ratios were plotted for each DMSO concentration against the indicated concentrations of 9-cis-retinoic acid (n=8 for each data point).

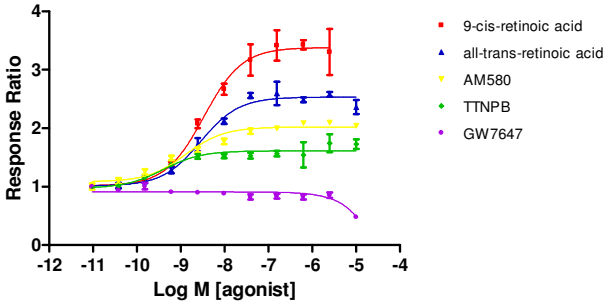
Dose Response with additional nuclear receptor agonists

Figure 8a – Dose response of additional nuclear receptor agonists.



RXR beta-UAS-*bla* HEK 293T cells (10,000 cells/well) were plated the day of the assay in a 384-well black-walled CellBind plate. Cells were stimulated with a serial dilution series of 9-cis-retinoic acid (Biomol cat# GR101), TO901317 (Calbiochem cat# 575310), or T3 (thyroid hormone 3,5,3'-triiodothyronine) (Calbiochem cat# 642511) in the presence of 0.5% DMSO for 16 hours at 37ºC. Cells were loaded for 90 minutes with LiveBLAzer™-FRET B/G Substrate (1 µM final concentration of CCF4-AM). Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader in bottom-read mode, and the background-corrected Response Ratios were plotted against the indicated concentrations of agonists (n=8 for each data point).

Figure 8b – Dose response of additional nuclear receptor agonists.



RXR beta-UAS-*bla* HEK 293T cells (10,000 cells/well) were plated the day of the assay in a 384-well black-walled CellBind plate. Cells were stimulated with a serial dilution series of 9-cis-retinoic acid (Biomol cat# GR101), all-trans-retinoic acid (Sigma cat# R2625), TTNPB (Sigma cat# T3757), GW7674 (Sigma cat# G6793), or AM580 (Sigma cat# A8843) in the presence of 0.5% DMSO for 16 hours at 37ºC. Cells were loaded for 90 minutes with LiveBLAzer™-FRET B/G Substrate (1 µM final concentration of CCF4-AM). Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader in bottom-read mode, and the background-corrected Response Ratios were plotted against the indicated concentrations of agonists (n=8 for each data point).

Assay performance with variable assay plate types

Figure 9a. – 9-cis-retinoic acid dose response with 20K, 10K, 5K, and 2.5K cells/well in a poly-d-lysine assay plate.

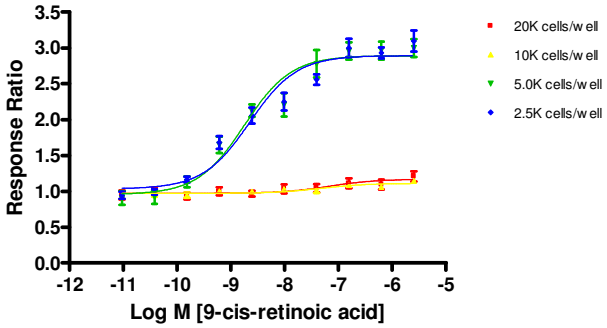


Figure 9b. – 9-cis-retinoic acid dose response with 20K, 10K, 5K, and 2.5K cells/well in a CellBind assay plate.

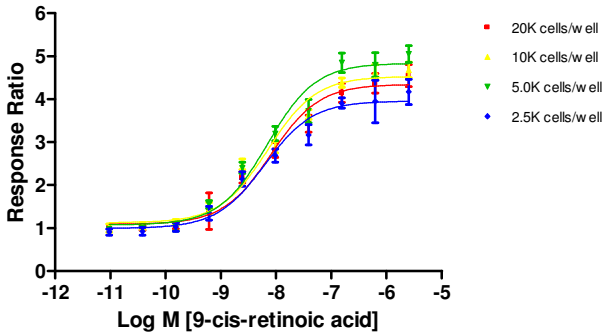
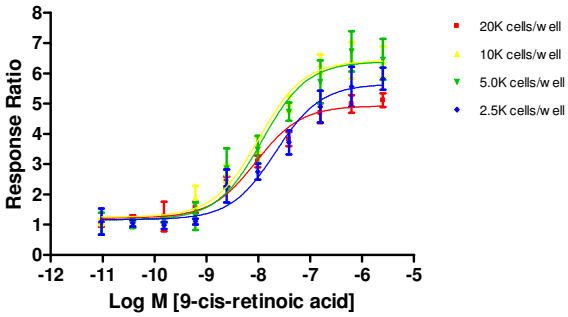


Figure 9c. – 9-cis-retinoic acid dose response with 20K, 10K, 5K, and 2.5K cells/well in a tissue culture treated assay plate.



RXR beta-UAS-*bla* HEK 293T cells were plated the day of the assay at 20,000, 10,000, 5,000, or 2,500 cells/well in either a 384-well black-walled (a) poly-d-lysine, (b) CellBind, or (c) tissue culture treated assay plate. Cells were stimulated with a serial dilution series of 9-cis-retinoic acid (Biomol cat# GR101) in the presence of 1.0% DMSO for 16 hours at 37ºC. Cells were loaded for 90 minutes with LiveBLAzer™-FRET B/G Substrate (1 µM final concentration of CCF4-AM). Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader in bottom-read mode, and the background-corrected Response Ratios were plotted against the indicated concentrations of agonists (n=8 for each data point.

Dose Response with additional nuclear receptor agonists in the presence and absence of RXRα antagonist RO41-5253

Figure 10a. – Dose response of 9-cis-retinoic acid in the presence or absence of RO41-5253

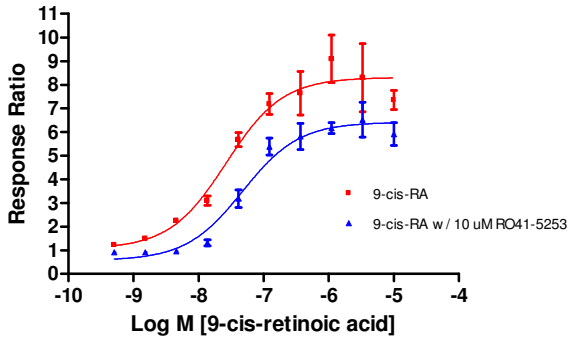


Figure 10b. – Dose response of all-trans-retinoic acid in the presence or absence of RO41-5253

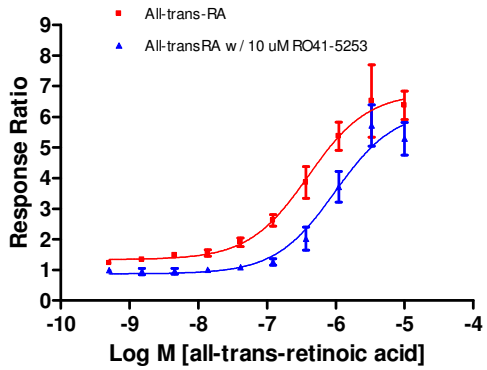


Figure 10c. – Dose response of AM580 in the presence or absence of RO41-5253

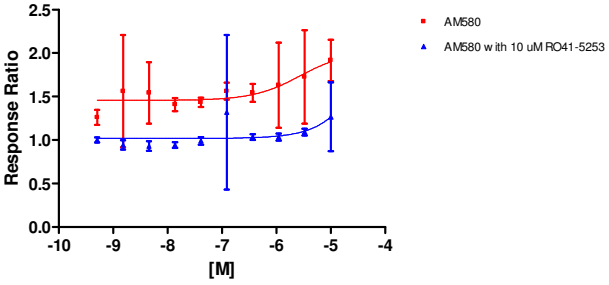
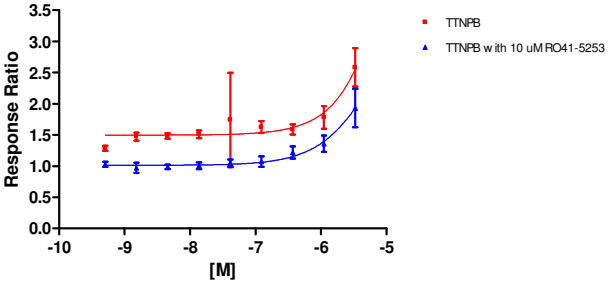


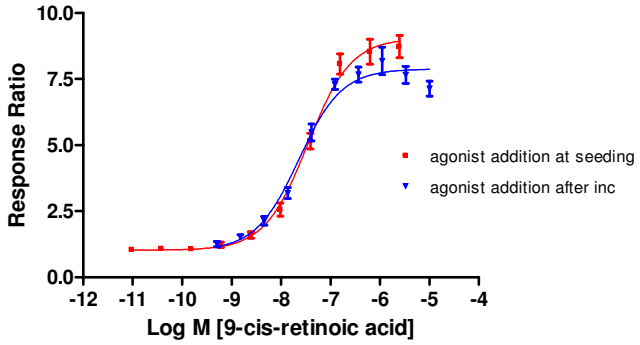
Figure 10d. – Dose response of TTNPB in the presence or absence of RO41-5253



RXR beta-UAS-*bla* HEK 293T cells (10,000 cells/well) were plated the day of the assay in a 384-well black-walled CellBind plate. Cells were treated with and without 10 µM RO41-5253 (Biomol cat# G110) in the presence of 1% DMSO for 30 minutes at 37°C. Cells were then stimulated with a serial dilution series of 9-cis-retinoic acid (Biomol cat# GR101), all-trans-retinoic acid (Sigma cat# R2625), TTNPB (Sigma cat# T3757), or AM580 (Sigma cat# A8843) for 16 hours at 37ºC. Cells were loaded for 90 minutes with LiveBLAzer™-FRET B/G Substrate (1 µM final concentration of CCF4-AM). Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader in bottom-read mode, and the background-corrected Response Ratios were plotted against the indicated concentrations of agonists (n=8 for each data point).

Effect of pre-incubating cells on assay performance

Figure 11. – Dose response of 9-cis-retinoic acid upon seeding of cells or after >3 hr pre-incubation



RXR beta-UAS-*bla* HEK 293T cells (10,000 cells/well) were plated the day of the assay in a 384-well black-walled CellBind plate. After either a 0 hour or >3 hour pre-incubation at 37°C, the cells were stimulated with a serial dilution series of 9-cis-retinoic acid (Biomol cat# GR101) in the presence of 1.0% DMSO for 16 hours at 37°C. Cells were loaded for 90 minutes with LiveBLAzer™-FRET B/G Substrate (1 µM final concentration of CCF4-AM). Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader in bottom-read mode, and the background-corrected Response Ratios were plotted against the indicated concentrations of agonists (n=8 for each data point).

References

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