INDIGO BIOSCIENCES
Nuclear Receptor & *In Vitro* Toxicology Solutions™

INDIGlo Luciferase Detection Reagent
For Cell-based Reporter Assays

Description

INDIGlo Luciferase Detection Reagent (LDR) is formulated to deliver steady, long-lived luminescence signal from mammalian reporter cells expressing firefly luciferase. For convenience, the reagent is provided 'ready to use', without the requirement to first re-constitute powder-form components.

INDIGlo LDR works equally well in quantifying expressed luciferase activity from 96- or 384-well format reporter assays. The resulting light emission is stable over a 2 hr period (**Figure 1**), thereby making INDIGlo LDR an ideal, economical detection reagent for both low- and high-throughput batch processing of assay plates.

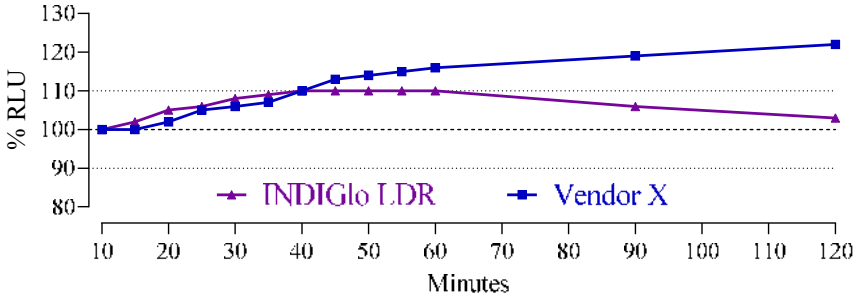


Figure 1. Steady light output from INDIGlo Luciferase Detection Reagent. HEK-293T cells expressing firefly *luc* were grown to confluence in a white cell culture treated 96-well assay plate, and luciferase activities were quantified using either INDIGlo LDR or a popular competitive steady-luminescence detection reagent (Vendor X). Relative Luminescence Unit (RLU) values are normalized as a percent of the initial RLU reading taken after a 10-minute post-dispensing rest period. Luciferase activity from the INDIGlo LDR treated reporter cells demonstrate superior stable light emission over a 2 hr assay period, with low (≤ 10%) fluctuation of signal.

INDIGlo LDR is formulated as a 2x-concentrated reagent, thereby allowing greater versatility in its use. The 2x-concentrated reagent will be used when using **384-well** or **1536-well** assay plates that are processed using a homogenous assay strategy (depicted in **Figure 2**).

Alternatively, when processing **96-well** assay plates either a homogenous or non-homogenous (**Figure 3)** assay strategy may be used. For convenience, this kit provides an optimized Dilution Buffer for use in diluting INDIGlo LDR when performing non-homogenous assays.

Regardless of one's assay setup preference INDIGlo LDR delivers rapid lysis of the reporter cells, robust luminescence signal, and sensitive quantification of expressed luciferase activities.

Alternative Assay Protocols

Homogenous Assays are performed by dispensing detection reagent directly into assay wells without first removing the culture media. This streamlined method is used when processing 384-well (or higher density) assay plates. Because INDIGlo LDR is formulated as a 2x-concentrated reagent the volume that is dispensed will be equal to the volume of culture media present in the assay wells.

The homogenous assay strategy may also be applied to processing 96-well assay plates, and this may be the preferred method if processing large numbers of plates.

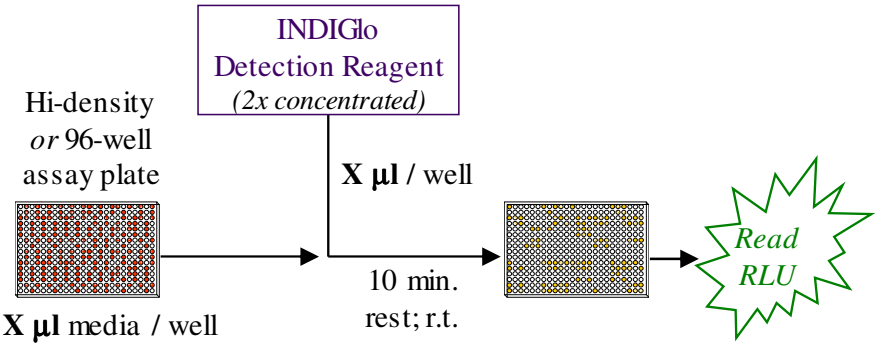


Figure 2. Homogenous Assay plate processing. Undiluted INDIGlo LDR is added directly into assay wells without prior media discard or wash steps. Dispense a volume of INDIGlo LDR that is equivalent to the volume of cell culture media in each well. Allow the plate to rest for 10 minutes, then quantify RLU values using a plate-reading luminometer.

Non-Homogenous Assays are performed by first removing the culture media from the assay plate, then dispensing INDIGlo LDR that has been pre-diluted (1:1) with the provided Dilution Buffer. This assay strategy performs very well, and its use is recommended by INDIGO when processing a small number of 96-well assay plates. Two advantages may be gained when using a non-homogenous assay protocol: *1.)* higher RLU values, and often improved EC50 values, are commonly observed (**Figure 4**). By first removing media from the assay wells the user eliminates unknown chemical variables that may otherwise impact the luciferase reaction chemistry. For example, some media components can adversely affect the activity of luciferase and/or assay sensitivity. Also, inhibitors of luciferase are sometimes encountered during small-molecule screens. This is an important consideration when users screen uncharacterized test compounds at high, and varying, treatment concentrations. Finally, *2.)* in terms of the number of assay plates that can be processed per volume of INDIGlo LDR, the non-homogenous assay format is typically the more economical choice.

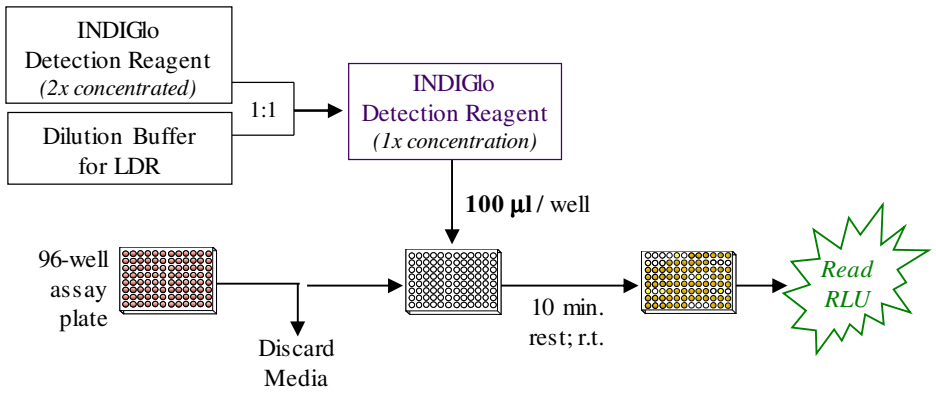
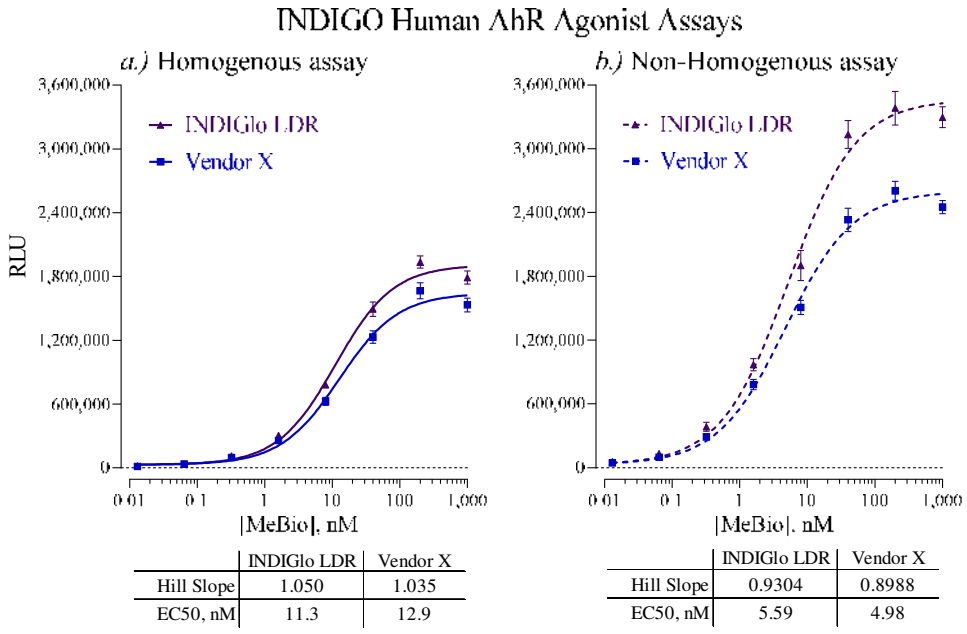


Figure 3. Non-Homogenous Assay plate processing. Combine equal volumes of INDIGlo LDR and LDR Dilution Buffer to generate a 1x-concentration detection reagent. Culture media is then removed from the assay wells and 100 µL/well of the diluted INDIGlo LDR is dispensed. After a 10 minutes plate rest quantify RLU values.

INDIGlo LDR Performance



	INDIGlo LDR	Vendor X
Hill Slope	1.050	1.035
EC50, nM	11.3	12.9

	INDIGlo LDR	Vendor X
Hill Slope	0.9304	0.8988
EC50, nM	5.59	4.98

Figure 4. INDIGlo LDR performance from both Homogenous and Non-Homogenous assays. Dose-response analyses of Human AhR (INDIGO #IB06001, 96-well assay) was performed using the reference agonist MeBIO. AhR reporter cells were plated across two white assay plates, MeBIO treatment media were added, and the assay plates incubated for 24 hr. Plates were then processed with luciferase detection reagents in either *a.)* a homogenous or *b.)* a non-homogenous assay format. Resulting assay metrics are also shown from using another extended-glo detection reagent (Vendor X)**.**

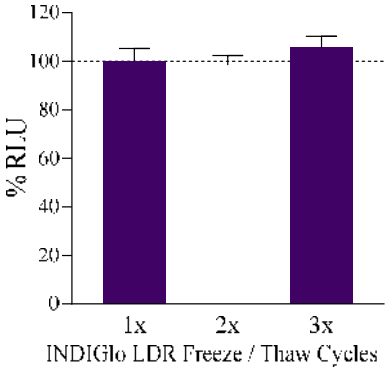


Figure 5. INDIGlo LDR potency is stable following repeated freeze and thaw cycles.

INDIGlo LDR was frozen at -20°C and thawed either 1, 2 or 3 times, then used to quantify luciferase activity from HEK-293:*luc* cells cultured in a white 96-well assay plate. No loss of reagent potency is observed between these conditions.

Reagent Components and Storage Conditions

INDIGlo Luciferase Detection Reagent kits provide two reagents in equal volumes:

- **INDIGlo LDR**, formulated as a 2x-concentrated reagent
- **Dilution Buffer**, for optional use if performing non-homogenous assays

INDIGlo LDR is packaged in amber tubes, sealed under argon, then frozen. Upon receipt, both reagents should be further stored at -20°C. INDIGlo LDR is stable for at least 6 months from the date of its manufacture; the expiration date is printed on the Product Qualification Insert that is enclosed in each kit.

After INDIGlo LDR has been thawed for the first time, it may be subjected to an additional three freeze / thaw cycles without loss of potency (**Figure 5**). If more than three thaw-cycles are anticipated, it is advised to dispense the first-time thawed reagent into smaller single-use aliquots, which are then stored frozen.

Reagent Preparations

For convenience, users may retrieve **INDIGlo LDR** and **Dilution Buffer** from freezer storage and place them in a dark refrigerator (+4°C) to thaw overnight. Approximately 30 minutes before intending to add the detection reagent to the cells, remove INDIGlo LDR and Dilution Buffer from the refrigerator and place them in a low-light area so they may equilibrate to room temperature. For non-homogenous assays, combine equal volumes of INDIGlo LDR and Dilution Buffer; mix gently to avoid foaming.

Note 1: Do NOT actively warm the INDIGlo LDR above room temperature. If this reagent was not allowed to thaw overnight in a refrigerator, a room temperature water bath may be used to expedite thawing.

Note 2: INDIGlo LDR is formulated with a minimum concentration of DTT, a low-volatility chemical to which some people have a heightened sensitivity.

Products

INDIGlo Luciferase Detection Reagent Kits

Product #	Product Descriptions	* Number of 96-well assay plates processed	
		Homogenous assay	Non-Homogenous assay
LDR-10	10 mL each of 2x-concentrated INDIGlo LDR and Dilution Buffer.	1 plate	2 plates
LDR-25	25 mL each of 2x-concentrated INDIGlo LDR and Dilution Buffer.	2.5 plates	5 plates
LDR-50	50 mL each of 2x-concentrated INDIGlo LDR and Dilution Buffer.	5 plates	10 plates
LDR-500	10x 50 mL each of 2x-concentrated INDIGlo LDR and Dilution Buffer.	10x 5 plates	10x 10 plates
Bulk volumes of INDIGlo LDR can be manufactured and packaged at any scale to meet Customers' specific requirements. Please Inquire.			

* Assumptions used for *Homogenous* assay setup: 96-well assay plates contain 100 µL/well of cell culture media, therefore, 100 µL/well of undiluted INDIGlo LDR is to be dispensed. Thus, 10 mL of INDIGlo LDR is sufficient to process one 96-well assay plate.

Non-Homogenous assay setup: 10 mL INDIGlo LDR is diluted (1:1) with 10 mL Dilution Buffer. Culture media is removed from the 96-well assay plate and 100 µL/well of the diluted INDIGlo LDR is dispensed. Therefore, a 10 mL vial of INDIGlo LDR is sufficient to process two 96-well assay plates.

Limited Use Disclosures

Products offered by INDIGO Biosciences, Inc. are for RESEARCH PURPOSES ONLY – not for therapeutic, diagnostic, or contact use in humans or animals.

Product prices, availability, specifications, and claims are subject to update without prior notice.

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