

# iLite® FAQ

This page answers some of the questions you may have about iLite® cell-based assays and our assay ready cell lines.

## Substrate

**Q: How long can the Bright Glo substrate be stored and at what temperature after re-constitution?**

A: Our preferred supplier, Promega, recommends to store the re-constituted product at -70°C for up to one month

**Q: How long can the Bright Glo substrate be left after addition to the plate before reading?**

A: The signal from the Bright Glo substrate decreased quite rapidly so we recommend reading the plate 2 minutes after addition of the substrate to the plate. Some studies carried out to determine the effect over time has shown that the decrease in signal over time was approximately 10% after 5 minutes, 20% after 10 minutes and 60% after 30 minutes.

## Normalization

**Q: How does normalization using the normalization reporter gene work in the iLite assays?**

A: Most of the iLite cell lines from Euro Diagnostica have two luciferases incorporated, one which is connected to a specific receptor activation (e.g. Firefly) and a second which runs under the control of a constitutive promoter (e.g. Renilla). The signal from the latter will be proportional to the cell activity, and can thus be used to compensate for non-specific effects, such as serum matrix effects. The results are normalized by simply dividing the signal from the specific readout with that of the normalization readout.

**Q: Can I just use the Firefly luciferase and keep the normalization readout silent? And when is this an advantage?**

A: Yes, you can choose to read either both or just one of the luciferase signals, depending on the substrate used. To just read the Firefly luciferase signal (the specific signal), the second substrate intended for reading the normalization signal is simply not added, thereby keeping the second luciferase silent. For more information, please consult the manufacture Promega's website

Keeping the normalization signal silent can be an advantage from an economical point of view, when the iLite cells are used to determine functional activity in a non-complicated matrix which does not impact the cells (such as e.g. a buffer system for lot release or screening of drug candidates).

## Instrument Requirements

**Q: Is a CO2 incubator required to run the iLite assays?**

A: Yes a humidified CO2 incubator (i.e. CO2 incubator with a tray of water in bottom shelf) is required to run all the iLite assays (5% CO2).

## Luminometer

**Q: What type of Luminometer is recommended for iLite assays?**

A: As the iLite® technology readout is based on luminescence, a sensitive luminometer capable of reading 96-well plates is needed. The following models work well with the iLite® technology

Model	Manufacturer
GloMax® Discover System	Promega
GloMax® Explorer System	Promega
SpectraMax L Microplate Reader	Molecular Devices
Perkin Elmer 1420 Victor Light	Perkin Elmer
Enspire®	Perkin Elmer

**Q: Can a luminometer with injectors be used to read assays with Dual Glo?**

A: The Dual-Glo® Luciferase Reagents are not designed for the use with the automated injectors that are integrated into some luminometers, as excessive reagent foaming may occur. Also as the Dual-Glo Luciferase Assay System which is used in our assay required a 10 minute incubation post addition of both the luciferase and the Stop and Glo, it would take a long time to read a plate if injectors were used.

**Q: Is it preferred to use flash or no flash on the luminometer?**

A: We do not recommend using flash, since it is very difficult to validate because of the high variability of this detection method.

**Q: What wavelength should I set the luminometer to?**

A: Luciferases generate visible light and with a broad range. We recommend that the wavelength that is measured is as broad as possible in the visible light area, therefore no restrictions in wavelength are needed. Most eukaryotic cells do not produce light, hence the background light from the iLite cells is very low and there is therefore no need to use any filter for the light emitted in a luciferase assay.

**Q: How can you measure two luciferases in one well?**

A: The readings are performed sequentially. The manufacturer Promega supplies a luciferase kit, which includes two substrates. First, the substrate for the Firefly luciferase is added, and after a short incubation the plate is read. After the plate has been read, the second substrate, which contains an agent that quenches the Firefly substrate, is added. After another short incubation, the plate is again read in the luminometer and the normalization signal is determined.

## Utilities

**Q: What type of plates are required for the iLite assays?**

A: A. The iLite assays can be run in the 96 well white plates e.g. from Perkin Elmer (Cat No. 6005680).

**Q: Can the reservoirs be re-used?**

A: No, only single use reservoirs should be used since detergents can interfere with the cells.

## Other

**Q: How are your reporter gene cell lines transfected?**

A: The iLite cell lines are stably transfected reporter gene cell lines. The transfection does not involve the use of any virus. The iLite cell lines are classified as class 1 Genetically Modified Microorganisms.

**Q: What causes the difference in colour in reagents/diluent some are light pink and other are yellow/orange, and will it affect my assay?**

A: The difference in colour in the reagents is due to a small variation in pH as a result of exposure to oxygen. This will not interfere with the assay as the pH of the reagents will adjust after a short period in the incubator.

**Q: Can the iLite IFN Type I Assay Ready Cells be used for distinguishing between IFN beta 1a and IFN beta 1b neutralizing antibodies (NABs)?**

A: No, NABs to both IFN beta 1a and beta 1b will be identified with these cells and hence the cells cannot distinguish between neutralising antibodies for one or the other. In addition, the iLite IFN Type I Assay Ready Cells can also detect Interferon Alpha.

**Q: Is it possible to culture the cells and prepare stocks of the iLite cells.**

A: No, the cells are not supplied for further culturing. The cells are for single use only.[2] Licensing options for culturing are available for certain iLite products. Please contact us if you want to learn more about this option.

**Q: What is the difference between the iLite assays and ELISA's (Ligand binding assays)**

A: ELISA assays measure the concentration of drug or anti-drug antibodies (ADA) by detecting their binding to specific antibodies coated on the plate. Since ELISA assays detect everything that binds, they cannot distinguish between active or inactive drug, or between ADAs and drug neutralizing antibodies (NABs). iLite cell based assays measure the functional activity of a drug or drug NABs, by detecting binding of a target to its receptor on the cell surface, which initiates intracellular pathways triggering transcription of the reporter gene. Neutralization of the target by the drug or by NABs will block it from binding to the receptor, thereby lowering the signal from the reporter gene. iLite assays can only detect NABs and not ADAs, since non-neutralizing ADAs will not block the drug from performing its mechanism of action.

**Q: What is the sensitivity of the potency or neutralizing antibody assays based on the iLite cell lines?**

A: As opposed to an ELISA, where one assay is developed for each drug, one iLite cell line can be used for any drug specific for that target. As an example, when using the iLite® TNF-alpha Assay Ready Cells, potency and immunogenicity of Infliximab, Adalimumab, Etanercept, Golimumab and Certolizumab pegol can all be measured using the same assay. The sensitivity of the assay will depend on the specific drug of interest and the concentration of that drug used in the assay. Therefore, the sensitivity of the assay needs to be established for each drug and the assay conditions applied.

**Q: Which GMM classification do the iLite reporter gene cell lines belong to?**

A: All of the iLite reporter gene cell lines are classified as GMM class 1.

*\*These products are intended for professional research use only. The data and results originating from using the products, should not be used either in diagnostic procedures or in human therapeutic applications.*

*In accepting delivery of iLite® Assay Ready Cells the recipient agrees not to sub-culture these cells, attempt to sub-culture them or to give them to a third party, and recipient is only to use them directly in assays. The iLite® cell-based products are covered by patents which are the property of Euro Diagnostica AB and any attempt to reproduce the delivered iLite® Assay Ready Cells would constitute an infringement.*

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