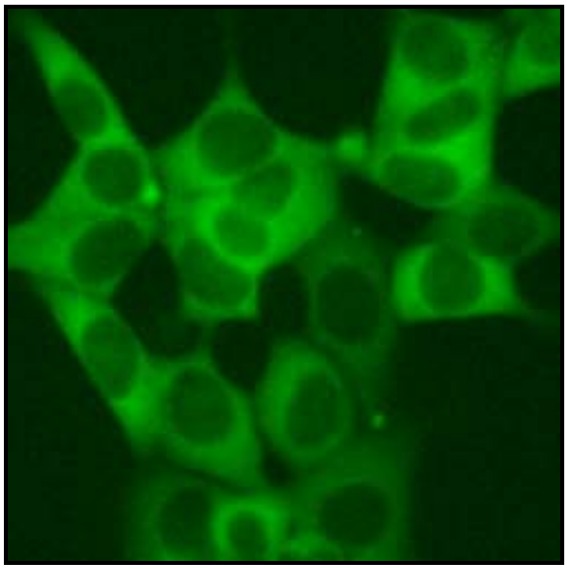
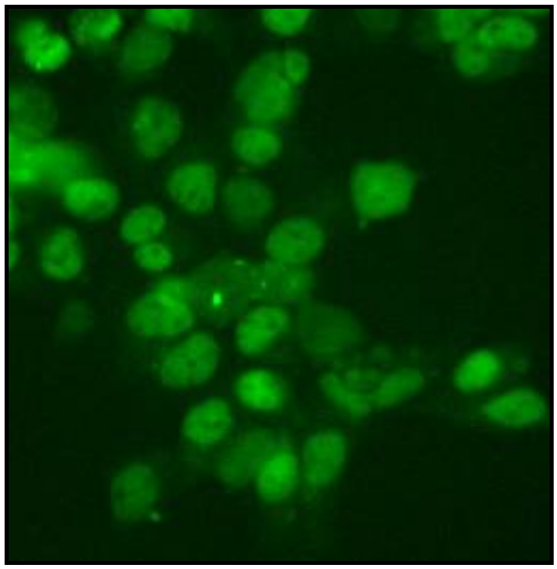
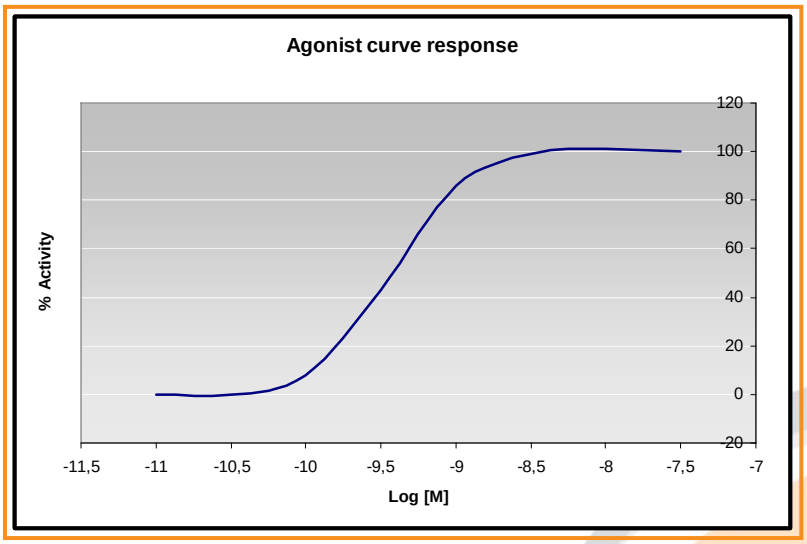
REF: P20114

NUCLEAR HORMONE RECEPTOR TRANSLOCATION ASSAYS

- FLUORESCENT HUMAN GLUCOCORTICOID RECEPTOR CELL LINE -







Product name: hGR-tGFP / HEK293 cell line

Ec_{50} Dexametasone: 6 x 10^{-10} M

Z´: 0.96+/- 0.01

VAT No. ESB95481909

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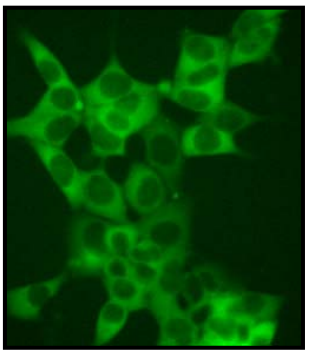
hGR NUCLEAR TRANSLOCATION ASSAY

HUMAN tGFP GLUCOCORTICOID RECEPTOR CELL LINE



Product Name: tGFP-hGR / HEK293

Official Full Name: Glucocorticoid receptor

DNA Accesion Number: GenBank NM_000176

Host Cell: HEK293

Format: Cryopreserved vials

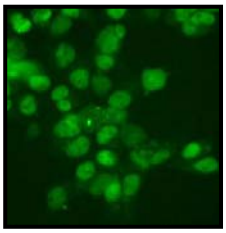
References:

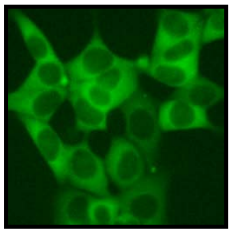
- ***P20114:*** 2 vials of 3 x 10^6 proliferative cells
- ***P20114-DA***: 1 vial of 2.5 x 10^6 division-arrested cells

Assay Briefly description

Each vial of P20114 contains HEK293 cells stably expressing human Glucocorticoid receptor (GR) tagged with tGFP.

Innoprot's GR Nuclear Translocation Assay cell line has been designed to assay compounds or analyze stimuli for their ability to modulate glucocorticoid receptor, following nuclear translocation process and quantifying the fluorescence distribution inside the cells.





This highly reproducible assay allows monitoring Glucocorticoid receptor nuclear translocation in High Content Analysis and fluorescence microscope applications.

Background

Glucocorticoid receptor, also known ***Nuclear receptor subfamily 3, group C, member 1, (NR3C1)***. The protein encoded by this gene is a receptor for glucocorticoids that can act as both a transcription factor and a regulator of other transcription factors. This protein can also be found in heteromeric cytoplasmic complexes along with heat shock factors and immunophilins. The protein is typically found in the cytoplasm until it binds a ligand, which induces transport into the nucleus. Mutations in this gene are a cause of glucocorticoid resistance, like cortisol resistance. Alternate splicing, the use of at least three different promoters and alternate translation initiation sites result in several transcript variants encoding the same protein or different isoforms, but the full-length nature of some variants has not been determined. (Ref: provided by RefSeq).

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Applications

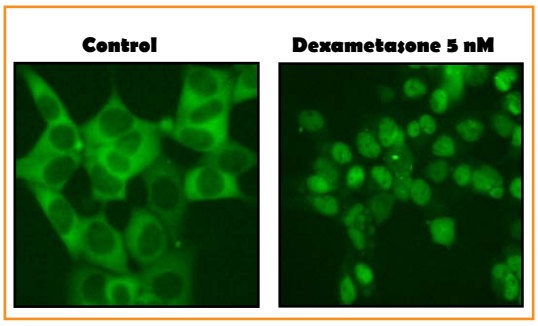
- Nuclear Trafficking assays
- Binding assays for GR
- Cellular Translocation Monitoring

Material Provided

- **P20114**: 2 vials of 3 x 10^6 proliferative cells
- **P20114-DA**: 1 vial of 2.5 x 10^6 division-arrested cells

Trafficking of tGFP-hGR

In the absence of dexametasone, glucocorticoid receptor is predominantly diffused through the cytoplasm. When dexametasone binds to GR, the receptor translocates and accumulates into the cell nucleus. The fusion proteins tGFP-hGR can be detected in live cells using an epifluorescence microscope.



Fluorescent hGR diffused through the cytoplasm

Fluorescent hGR accumulated into the cell nucleus

Assay Details

HEK293 cells stably expressing human glucocorticoid receptor tagged in the N-terminus with tGFP were stimulated with different concentrations of dexametasone during 16 hours. After that, the receptor translocated and fluorescence accumulated in the cell nucleus was detected by fluorescence using image analysis algorithms.

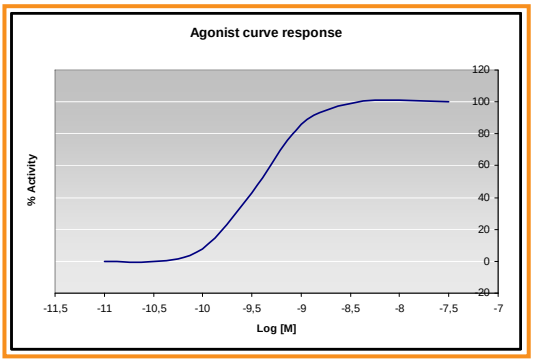


Fig.3. Dexametasone concentrations response in the hGR-tGFP nuclear translocation assay. Cells were treated with 4 log dilution series (n=4). The Ec50 for the dexametasone was ~ 0.6 nM after a treatment of 16h with agonist..

% Activity was calculated relative to positive (1uM). This hGR-tGFP nuclear translocation assay was validated with an average of **Z'= 0.96+/- 0.01 for High Content Screening**.

Use Restriction

This product contains a proprietary nucleic acid coding for a proprietary fluorescent protein intended to be used for research purposes only. No rights are conveyed to modify or clone the gene encoding fluorescent protein contained in this product, or to use the gene or protein other than for non-commercial research, including use for validation or screening compounds. For information on commercial licensing, contact Licensing Department, Evrogen JSC, email: license@evrogen.com

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