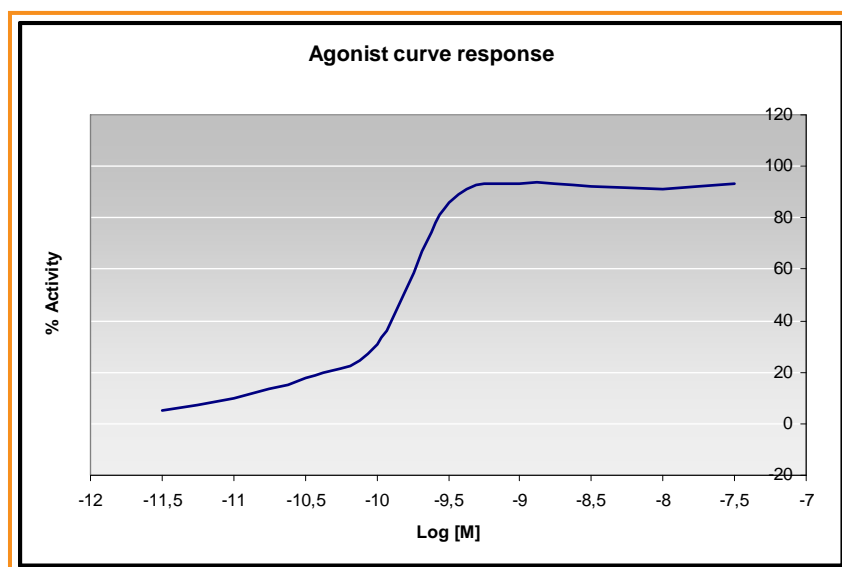
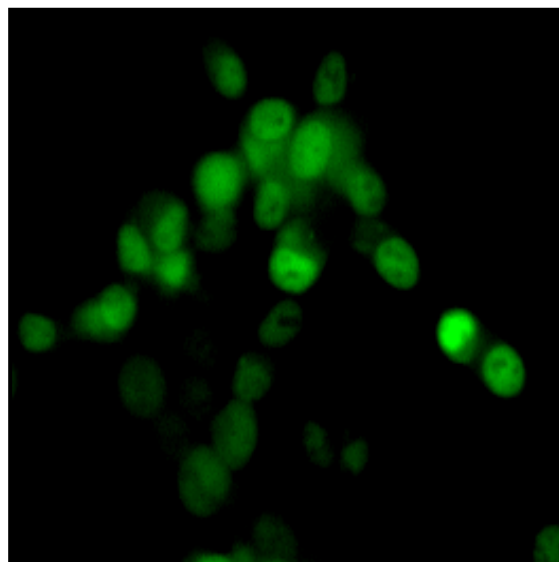
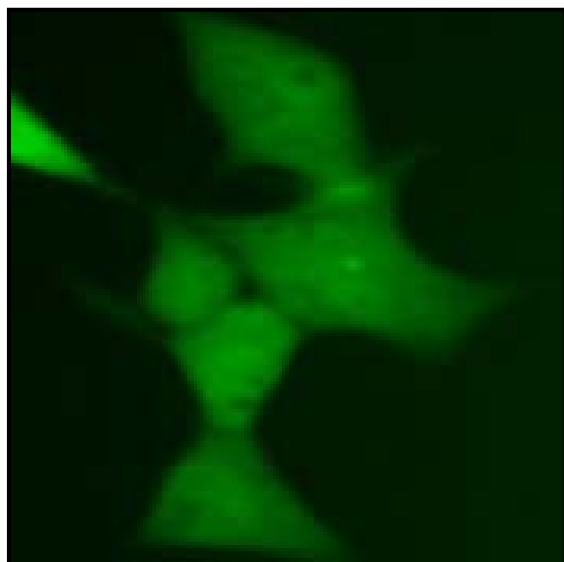


## NUCLEAR HORMONE RECEPTOR TRANSLOCATION ASSAYS

- FLUORESCENT HUMAN ANDROGEN RECEPTOR CELL LINE -



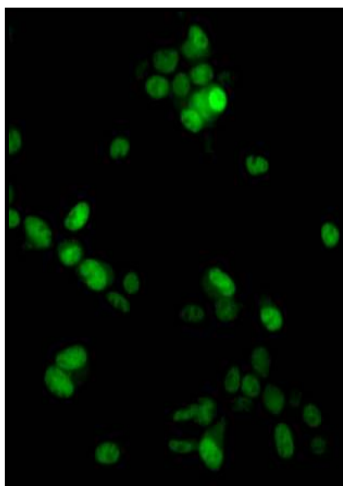
**Product name:** hAR-tGFP / HEK293 cell line

**EC<sub>50</sub> Testosterone:**  $2 \times 10^{-10}$  M

**Z':** 0.71+/- 0.03

## tGFP-hAR NUCLEAR TRANSLOCATION ASSAY

### HUMAN tGFP ANDROGEN RECEPTOR CELL LINE

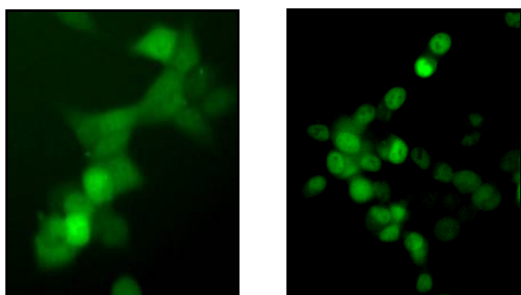


<b>Product Name:</b>	tGFP-hAR / HEK293
<b>Official Full Name:</b>	Androgen receptor
<b>DNA Accession Number:</b>	GenBank BC132975
<b>Host Cell:</b>	HEK293
<b>Format:</b>	Cryopreserved vials
<b>References:</b>	
	📄 <b>P20113:</b> 2 vials of $3 \times 10^6$ proliferative cells
	📄 <b>P20113-DA:</b> 1 vial of $2.5 \times 10^6$ division-arrested cells
<b>Storage:</b>	Liquid Nitrogen

#### 📄 **Assay Briefly description**

tGFP-hAR/HEK293 contains HEK293 cells stably expressing human Androgen Receptor (AR) tagged with tGFP.

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



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



#### 📄 **Background**

**Androgen receptor**, this gene is more than 90 kb long and codes for a protein that has 3 major functional domains: the N-terminal domain, DNA-binding domain, and androgen-binding domain. The protein functions as a steroid-hormone activated transcription factor. Upon binding the hormone ligand, the receptor dissociates from accessory proteins, translocates into the nucleus, dimerizes, and then stimulates transcription of androgen responsive genes. This gene contains 2 polymorphic trinucleotide repeat segments that encode polyglutamine and polyglycine tracts in the N-terminal transactivation domain of its protein. Expansion of the polyglutamine tract causes spinal bulbar muscular atrophy (Kennedy disease). Mutations in this gene are also associated with complete androgen insensitivity (CAIS). Two alternatively spliced variants encoding distinct isoforms have been described.

### **Applications**

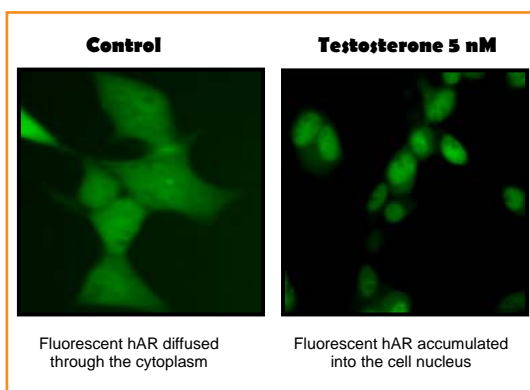
- Nuclear Trafficking assays
- Binding assays for AR
- Cellular Translocation Monitoring

### **Material Provided**

-  **P20113:** 2 vials of  $3 \times 10^6$  proliferative cells
-  **P20113-DA:** 1 vial of  $2.5 \times 10^6$  division-arrested cells

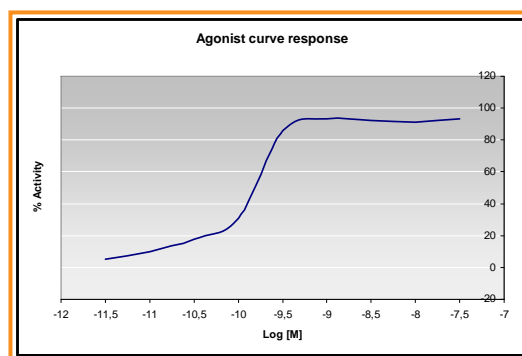
### **Trafficking of tGFP-hAR**

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



### **Assay Details**

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



**Fig.1. Testosterone concentrations; response in the hAR-tGFP nuclear translocation assay.** Cells were treated with 4 log dilution series (n=4). The  $EC_{50}$  for the testosterone was 0.2 nM after a treatment of 16h with agonist.

% Activity was calculated relative to positive (1 $\mu$ M). This hAR-tGFP nuclear translocation assay was validated with an average of  $Z' = 0.71 \pm 0.03$  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](mailto:license@evrogen.com)