



Nuclear Receptor Bio-Assays

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Joint Meeting of California and Water Reuse Research Foundation Project Teams
November 10, 2011

Agenda

- Who are we
- NR Portfolio Overview
- Specific Assays and Data
- Prior Environmental Toxicity Studies
- Water Testing Application Scenarios



Who We Are

Life Technologies – Carlsbad, CA / Corporate Headquarters



Globally rendering discoveries and developments that make life better

Corporate Fast Facts	
Presence	160 Countries
2010 Net Sales	\$3.6B
Employees	~ 11,000
Product Types	> 50,000
Water Saved 2008 vs 2010	25%



**Corporate Responsibility Magazine
“100 Best Corporate Citizens” List**

Life Technologies – Madison, WI Location



- Serves Bio/Pharma Drug Discovery with validated assays and services
- Developed and manufactures Life's nuclear receptor assay portfolio



**ISO 9001 Quality
Certification since 2008**



**OSHA's highest rating
of safety practices**



**Awarded ToxCast Contract
August 2011**



Implementation Support



Field Support Network

- Technical Sales Specialists (TSS)
- Field Application Specialists (FAS)
- Regionally located



Centralized Technical Support Team

- Dedicated support to our portfolio
- Staffed with Ph.D. scientists
- Phone and email support



Custom Development Services

- Our experts solving your challenges
- Dedicated team of scientists and project managers
- Proven track record since 2005



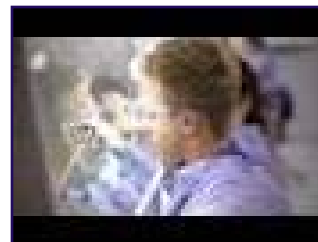
SelectScreen™ Profiling Services

- Our HTS lab instead of yours
- Automation and personnel providing fast, accurate and consistent results



Technical Seminars/Webinars

- Scientist-to-scientist engagement



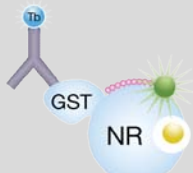
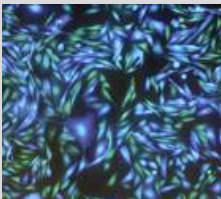

Custom Knowledge & Skill Training

- Build assay competency efficiently
- Lecture and hands-on training application
- E.g. - Hepatocyte Expert Program

We provide a spectrum of support options to meet customer needs



Our Nuclear Receptor Platform

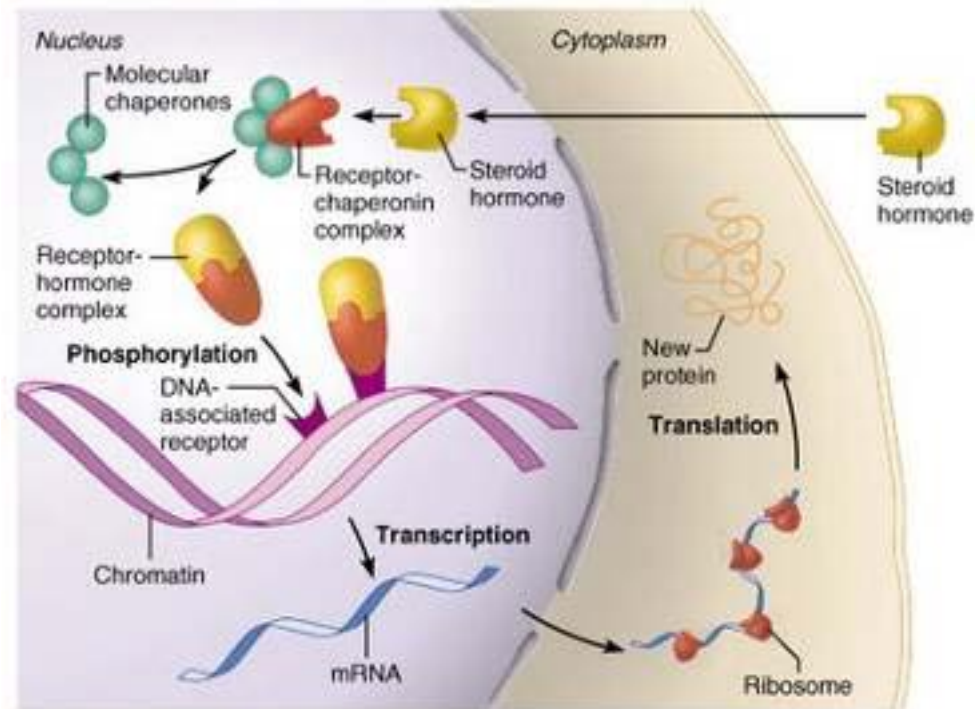
Question It Answers	Assay	Type	Features / Benefits	Available As: Product Service
<i>Does the compound bind and potentially affect NR function?</i>	LanthaScreen® TR-FRET Competitive Binding Assays	 <p>>30 Biochemical NR Screening Assays</p>	<ul style="list-style-type: none"> • Detects if compound binds to NR • Simple mix and read – no cell handling skills required 	✓
<i>What is the compound's functional impact?</i>	GeneBLAzer® Cell-Based Nuclear Receptor Assays	 <p>>20 Functional NR Cell-Based Assays</p>	<ul style="list-style-type: none"> • Quantitatively measures cell response • Elucidate agonist or antagonist effect • Option for “Assay Ready” cells 	✓ ✓
	SelectScreen™ NR Profiling Service	 <p>High Throughput Screening Service</p>	<ul style="list-style-type: none"> • Easiest of all – we do it for you! • Utilizes our functional cell-based assays 	✓

Broad options available for evaluating Nuclear Receptor impact



Nuclear Receptors

- 48 known human proteins
- Agonist binding to a nuclear receptor results in a conformational change in the receptor which activates the receptor
- Transcription factors together with coregulators to control the expression of genes important for
 - Development
 - Homeostasis
 - Metabolism
- Six steroid hormone receptors for sensing the presence of hormones and endocrine disrupting compounds



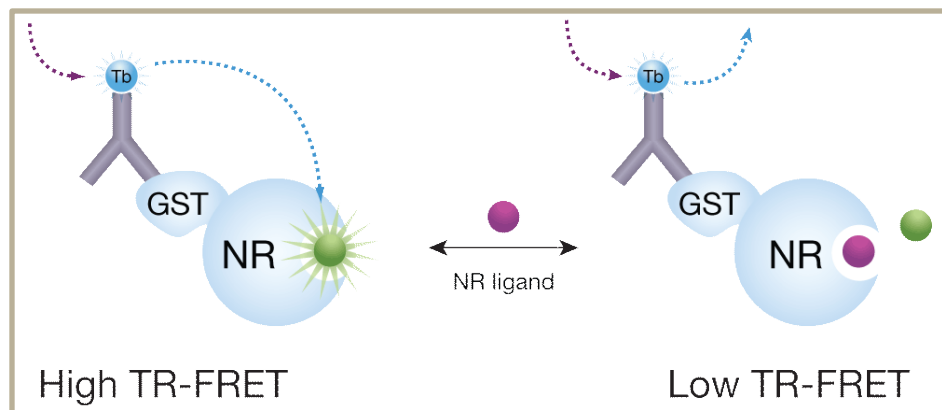
Copyright © 2001 Benjamin Cummings, an imprint of Addison Wesley Longman, Inc.

Disruption of normal NR action can cause significant functional changes



Biochemical Assays:

NR LanthaScreen[®] TR-FRET Competitive Binding



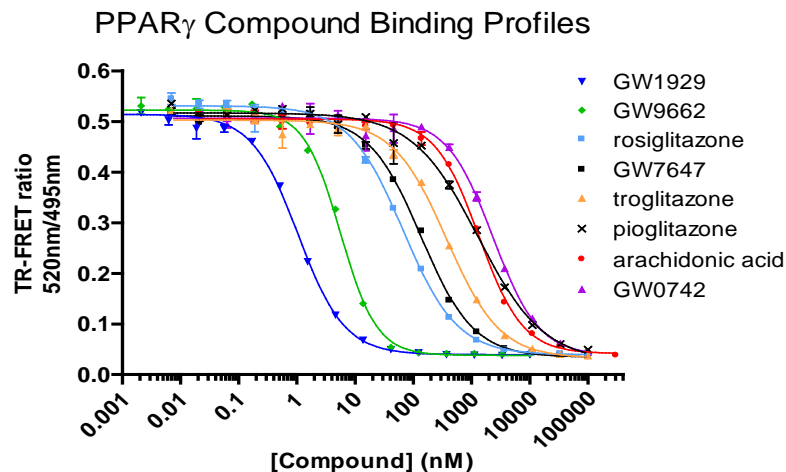
Key Features:

- Detects compound binding (cannot determine agonists and antagonists)
- Simple mix and read format
- TR-FRET to minimize optical interference from fluorescent compounds or particulates
- High throughput screening (HTS) compatible

Assay Configurations of Interest:

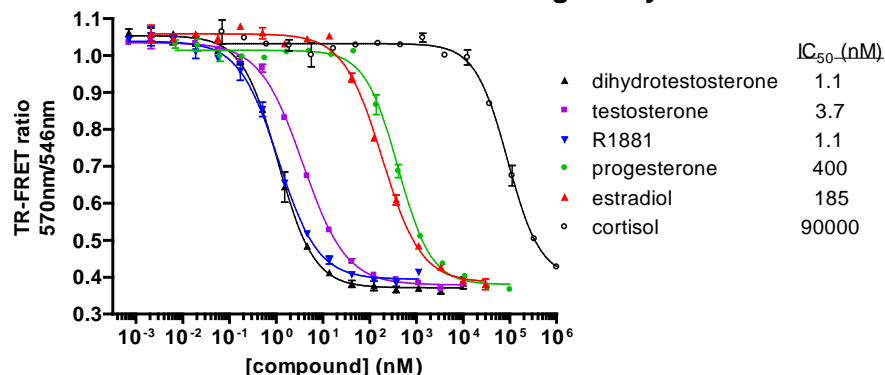
- Kits for: ER α , ER β , GR, PXR, PPAR α , PPAR δ , PPAR γ
- Tool box reagents for: AR, PR, VDR

**Simple format providing “Yes/No” impact potential,
no cell handling skills required**

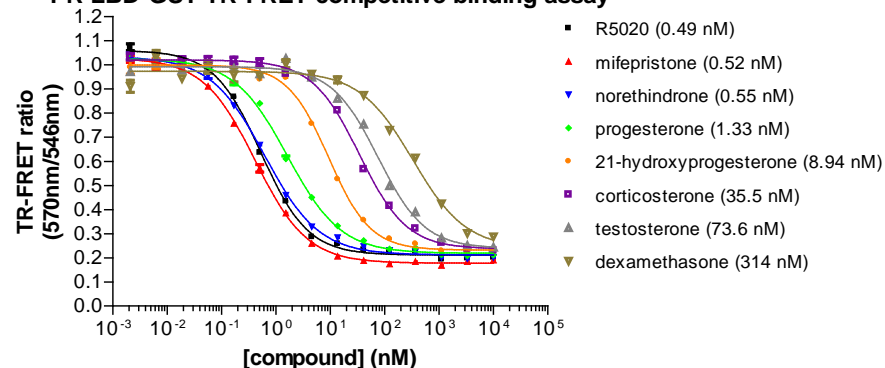


Profiles of Known Compounds for AR, PR, ER α and ER β

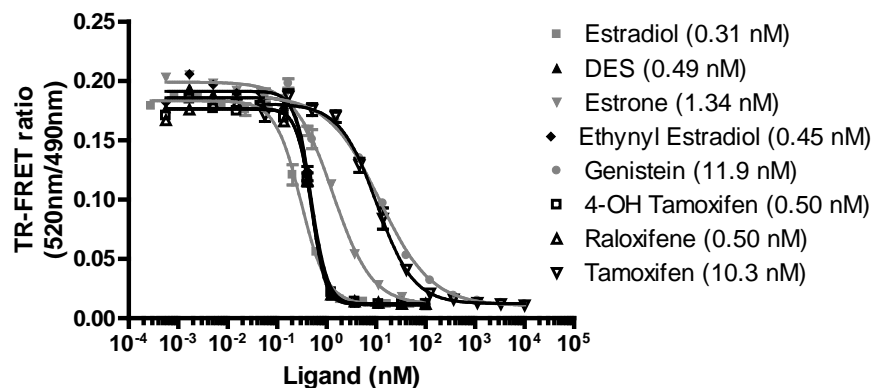
AR LBD-GST TR-FRET Binding Assay



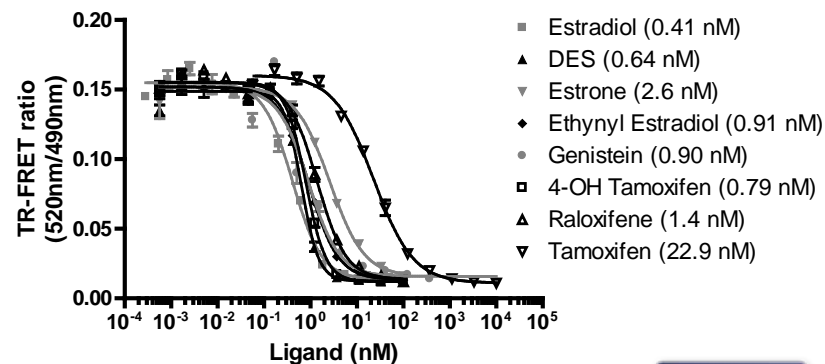
PR LBD-GST TR-FRET competitive binding assay



ER α LBD-GST TR-FRET Binding Assay



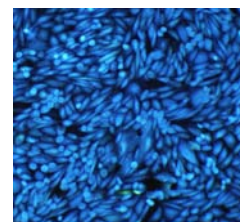
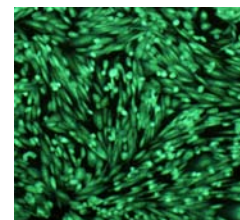
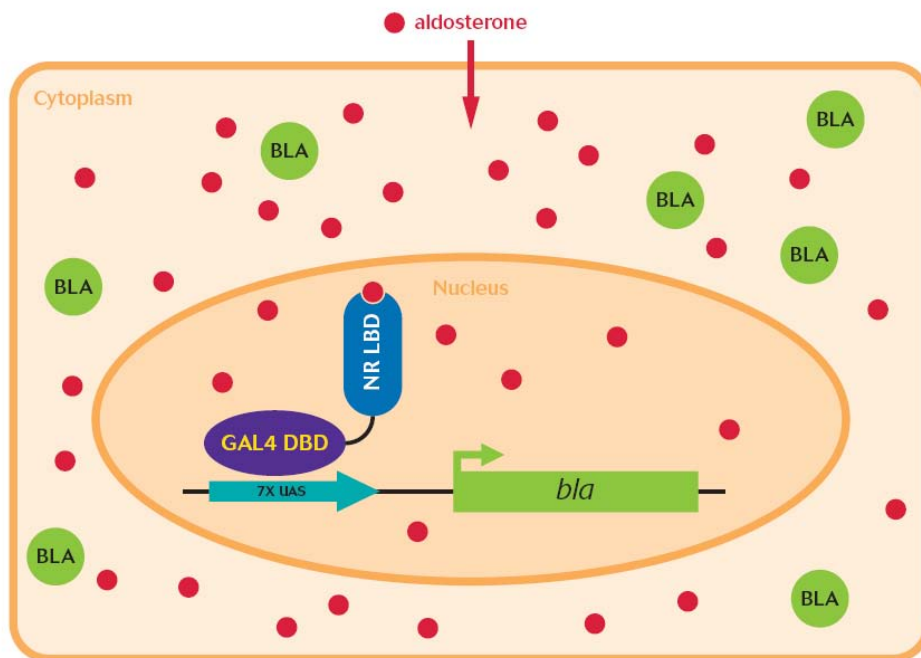
ER β LBD-GST TR-FRET Binding Assay



Sensitive assays – detect compounds with wide range of affinity



GeneBLAzer® Cell-Based Nuclear Receptor Assays



Advantages:

- Quantitatively measures compound's functional impact on cells
- Lower background and less interference from other NRs & crosstalk
- Functional read-out for ligand and receptor interaction

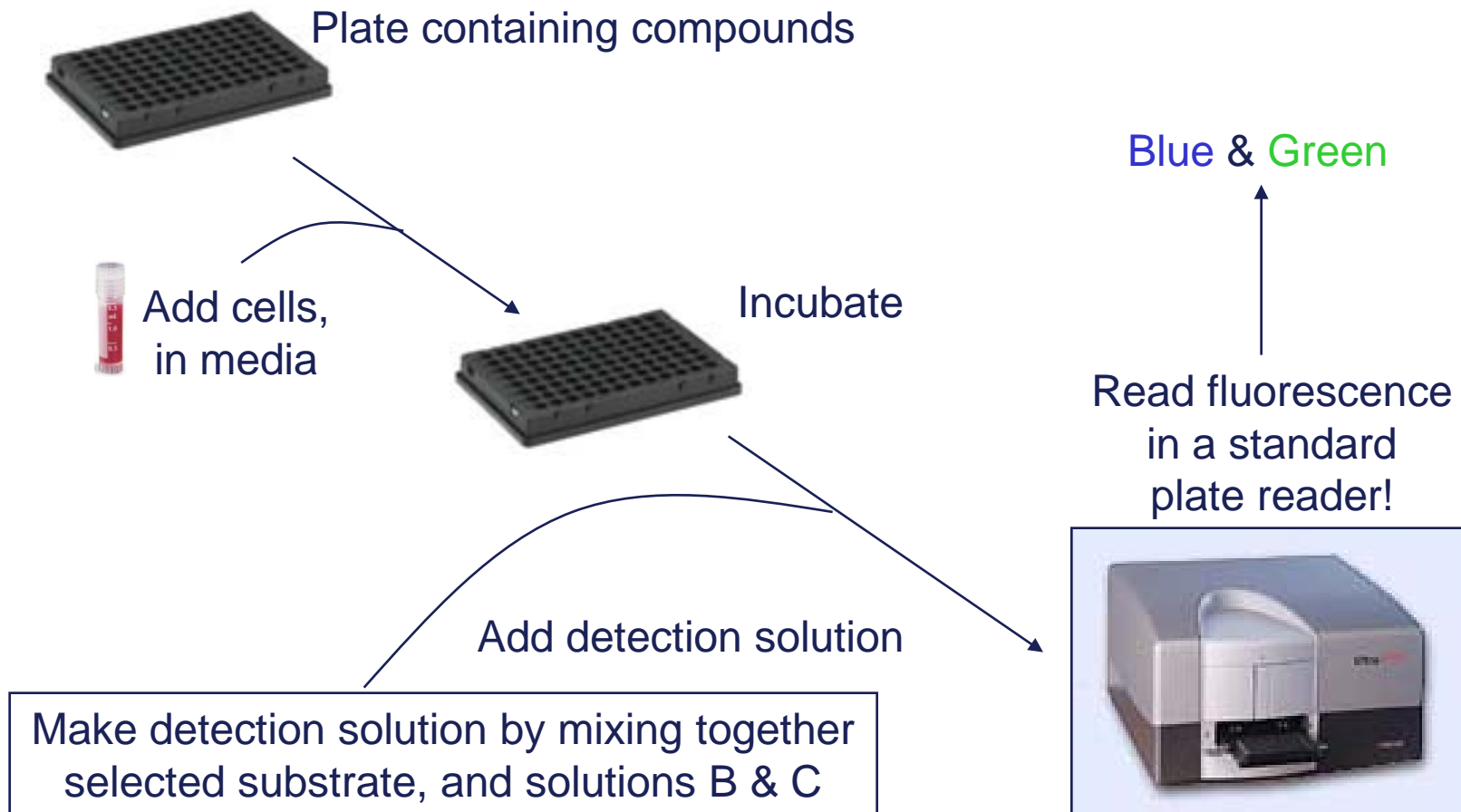
Available Formats:

- Cryopreserved stable cell lines
 - For expansion and continuous culture
- Cryopreserved assay-ready cells
 - Thaw and plate cells – run assay

Quantitative functional live cell assay – compatible with HTS and toxicity multiplexing



How Do You Screen with Our NR Cellular Assays?



Simple, HTS-compatible workflow: plate, mix, add and read!

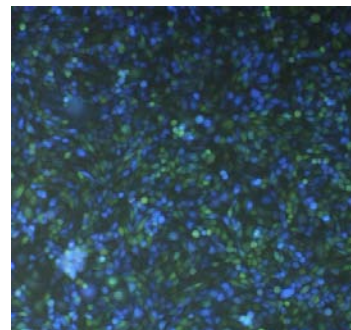


Multiplexing Functional and Cytotoxic Effects With ToxBLAzer™ Substrate

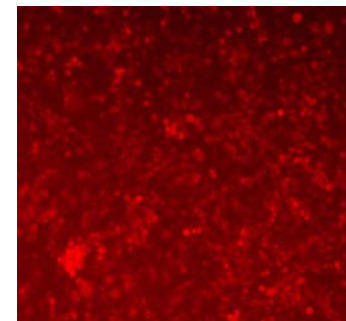
ToxBLAzer™ Use and Benefits:

- Simple – blends into functional assay's workflow; high throughput screening compatible
- Efficient – measure functional *and* cytotoxicity effects from the *same well at the same time*.
- Powerful – Identify false positives due to toxicity *and* assay errors that result in extremely low cell density.

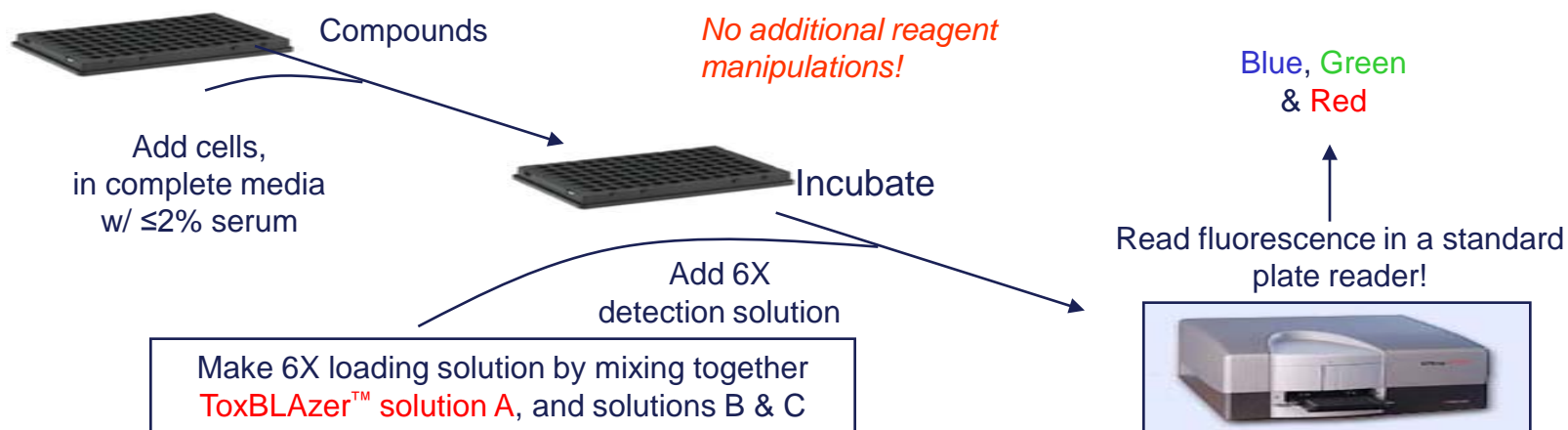
Functional Readout



Cytotoxicity Readout



Stimulated D1-CRE-*bla*-CHO-K1 Cells with ToxBLAzer™

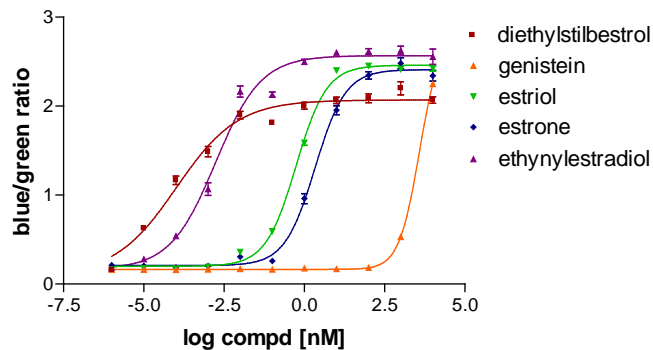


**Concurrently assess functional impact and flag cytotoxic effects.
Life also has a comprehensive suite of cell health assays.**

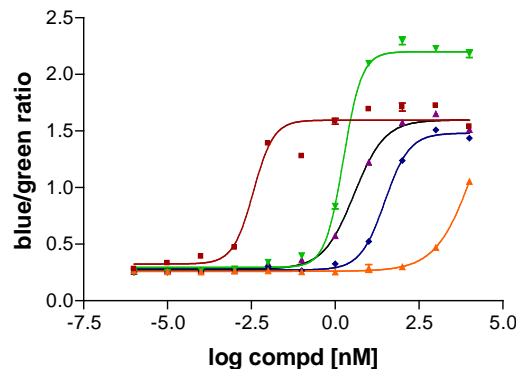


Cellular Assay Compound Profiles

ER α

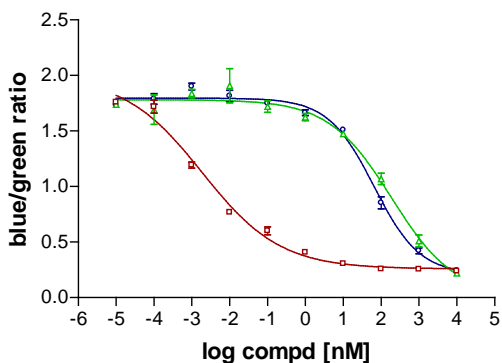
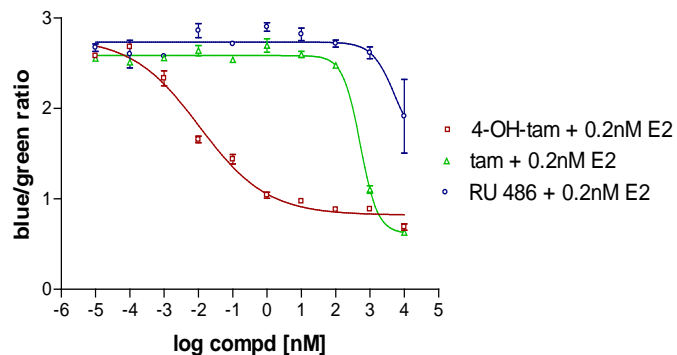


ER β



Agonists

Compound (EC ₅₀ , nM)	ER α	ER β
17 β -Estradiol	0.07	0.5
Diethylstilbestrol	0.002	0.0036
Genistein	3874	16275
Estriol	0.557	1.88
Estrone	2.175	30.34
Ethynylestradiol	0.0017	3.66



Antagonists

Compound (IC ₅₀ , nM)	ER α	ER β
4-OH-tamoxifen	0.011	0.002
Tamoxifen	521	189
RU-486	5217	64.5

For compound profiles for AR, PR, TR and PPAR γ , see appendix.

For additional compound profiles for ERs, see Wilkinson, et al. (2008) *Journal of Biomolecular Screening* 13:755.

Provides sensitive, quantitative functional assessment for agonists or antagonists



Applications of Our NR Cellular Assays in Environmental Toxicity Studies

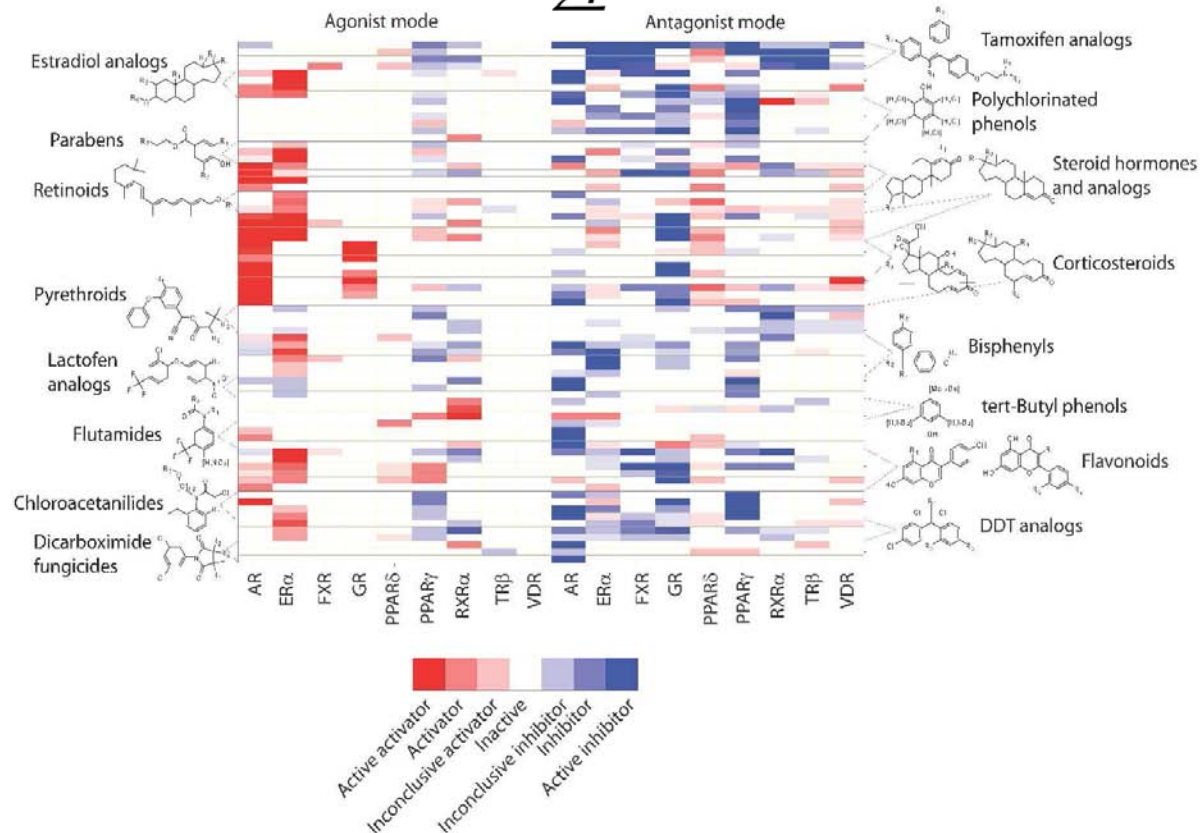


EPA National Center
for Computational Toxicology



NIH CHEMICAL GENOMICS CENTER

Tox21 Pilot Phase Study



Huang, et al. (2011) *Environmental Health Perspectives* 119:1142.

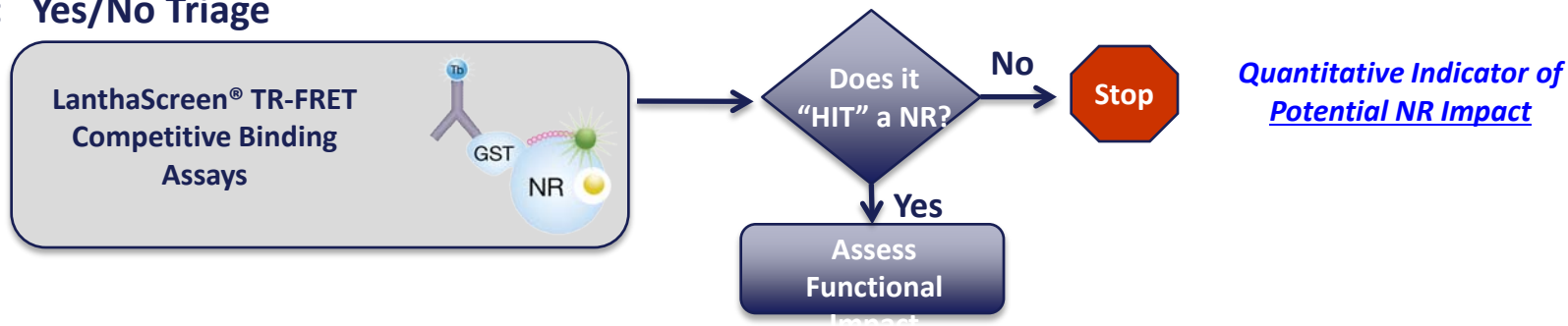
Prior Tox21 program evaluations demonstrated assay feasibility for environmental toxicity applications



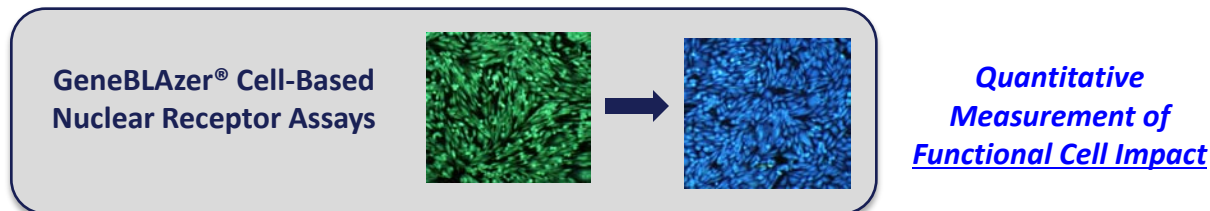
Water Quality Testing Scenarios with our NR Assays

Scenario 1: Multiple Pass – Triage and Hit Follow-Up

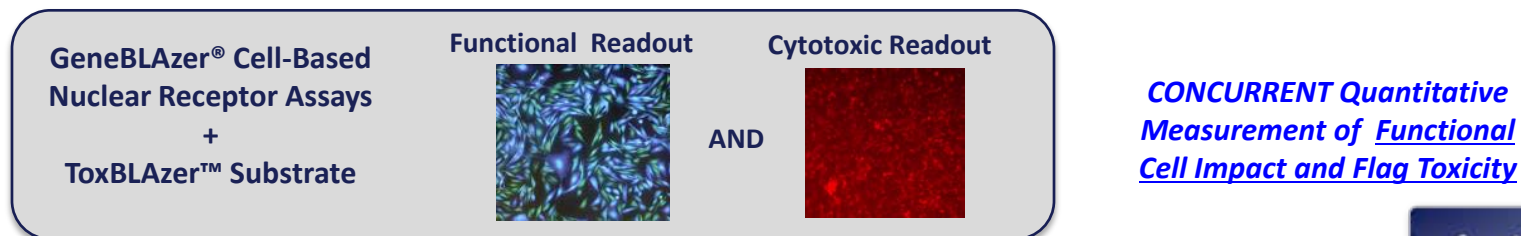
Pass 1: Yes/No Triage



Pass 2: Functional Impact Assessment



Scenario 2: One Pass – Functional Answer



Scalable, HTS compatible options for determining impact on nuclear receptors





Questions?

Thank you!

