

# Drug Discovery : HTS screening & toxicology

## Screening HTS reagents

### Technical tip

Interchim and Berthold collaboration supports further your works. Many of our fluorescence and luminescence reagents and kits were validated with



instruments, imaging systems.

\*Mithras LB940 MultiMode Reader



Includes a variety of technologies with samples injectors and robot integration module.

- ◆ Various formats (from Petri dishes to 1536 well plates)
- ◆ Absorbance
- ◆ Luminescence
- ◆ Fluorescence
- ◆ top and bottom measurement
- ◆ Polarisation (FP)
- ◆ FRET
- ◆ BRET
- ◆ AlphaScreen™
- ◆ High density and normal TRFluorometry see other absorbance, fluorescence and luminescence microplate readers page A366.

### Technical tip

#### HTS/Pharmacology screening methods

Screening is needed in drug discovery to analyse and select interesting compounds among all those generated (see page F13) by extraction procedures from natural materials, recombinant technologies, purification process, or synthetic combinatorial libraries.

Beside "in silico" prediction methods that are proposed to predict the physicochemical properties of biomolecules, their metabolism and their toxicity, **biological in vitro assays** are a key step to evaluate drug candidates real biological activity. Physical measurements also are useful to characterize compounds, refine pharmacological model and improve drug design and detection (generally more important at early stages). In vivo assays come generally only at the latest stages (pre-clinical: not for screening purpose).

Considering the amount of work up and downstream (years of testing for one drug), it is crucial to make the right choice of assays at the right development process. This is the endeavour of each lab in each pharmaceutical company. The choice and design of screening methods should also meet sometimes contradictory goals. Balancing cost, reliability and speed is a performance factor. The more numerous compound specimens are, the cheaper and faster testing should be. The more knowledge on drug biological activity once have, the more specific tests once have to choose.

## HTS kits and reagents (probes for cell assays)

Following reagents are available in special packaging and bulk quantities for HTS. Please inquire for jumbo prices. They include typically enzyme substrates for gene and protein reporter assays, and probes for cell biology testing in chromogenic or fluorescent detections. Descriptions are given in above sections.

### HTS screening methods and products :

Category -	Examples and types of products	More info
<b>cell assays -viability / cell proliferation</b>		
	probes for membrane integrity	page E146
	LDH assay #HT6340	page E155
	UptiBlue assay #669412	page E150
	CCK8 assay #899651	page E151
	ATPase assay #S2841C	page E153
	Accumax #UPN68091	page E30
<b>cell assays - Substrates for ATPases (ATP dependant enzymes, i.e. kinases)</b>		
	Coelenterazines	page E49
	ATPase assay #S2841C	page E153
<b>cell assays – Signaling Ca+, Hormones, Cytokines, Transcription factors...)</b>		
	Fluo-3. and other Indicators for ions	page E32, F15
	oestrone, IL, PAF, PGE2...	page E191, E206
	Pgd B2 # GK1640 & TBX E2 # AM1562 FPIA assays	page E206
<b>Cell assays - others</b>		
	MMP, ...	E145-E223
<b>Reporter assays-Cell lines, GFP, Enzyme Substrates</b>		
	X-Gal and other enzymatic substrates...	page D71
	A549 Cell Line (NFkB activity),...GFP, RFP	chapter B
<b>Immunoassays - microarrays</b>		
	EIA, FIA kits	chapter E
	Antibody microarrays	chapter B
	Primary antibodies	chapter A
	phage displays libraries	chapter B
<b>Enzyme assays</b>		
	Proteinase assay #HT1290	page E223
	Esterases, Caspases	page E160
	Clinical Biochemistry kits	page F4
	MPCR kits for cell biology (Cytokines, Apoptosis,...)	page D45
<b>Oxidative metabolism &amp; Toxicology assays</b>		
	ROS probes and assays	page E100
	Microsomes	page F18
	LHD assays #HT027	page F15
	CytoxiLux, GrantoxiLux (cell mediated toxicology)	page F16
<b>Protein characterization</b>		
	MS analysis / JBSscreen crystallisation solutions	chapter B
	Chromatography columns / Fluorescent derivatizations agents	chapter B

Following paragraphs essentially deals with primarily bioassays, including enzyme and cell based in vitro assays. **Bio-assays** should be designed carefully, to meet several requirements and give a very cost effective solution. Key parameters may vary at each level of the drug discovery development process (for pure samples for, cell or tissue samples). They include :

- ◆ **Choice of the target parameter** to be tested, may be the biological activity, a toxic effect, an interaction with a ligand (receptor, Ab...),...
- ◆ **Choice of a pertinent cell line or enzyme system**
- ◆ **Choice of a good technique**, adequate for the **sensitivity**, the **specificity**, the **ease of use**, suitability for **scale up** to numerous samples and eventually **automation**...

**End point assay** are generally useful in early screening, quantitative or **dynamic assay** may be preferred in drug or target characterisation steps, **multiplexed assays** are searched to save time and money while empowering data exploitation. **Flexibility** of processes may require optimization of the assay and adaptations.

Important probes to design HTS cell assays are listed there after.