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In vitro **Toxicology**

GreenScreen HC™ Genotoxicity Assessment

Background Information

'The *GADD45a*-GFP (GreenScreen HC™) reporter assay detects genotoxic damage in the human lymphoblastoid TK6 cell line and gives positive results for all classes of genotoxin, including mutagens, aneugens and clastogens.'

[3]Hastwell PW, Webster TW, Tate M, Billinton N, Lynch AM, Harvey JS, Rees RW and Walmsley RM (2009) *Mutagenesis* **24(5)**; 455-463

- Cyprotex have partnered with Gentronix, specialists in genotoxicity screening, to offer the GreenScreen HC™ assay.
- The GreenScreen HC™ genotoxicity assay utilises p53-competent human-derived TK6 cells to host the patented *GADD45a*-GFP reporter system. GADD45a has been implicated in the response to genome damage by genetic, biochemical and genomic approaches[1].
- The assay uniquely delivers both highly specific and highly sensitive detection of genotoxic stress in a human cell line.
- To detect 'pro-genotoxins' (i.e. chemicals which require metabolic activation to become genotoxic), the GreenScreen HC™ assay is performed both in the presence and absence of the post mitochondrial liver fraction, S9, typically prepared from chemically induced rat livers[2].
- GreenScreen HC™ offers several advantages over existing *in vitro* mammalian genotoxicity assays including ease of use, speed, improved accuracy and reduced compound requirements.

Protocol

Test Article Concentration
9 serial dilutions, typically 2-fold; e.g. 1000, 500, 250, 125, 62.5, 31.3, 15.6, 7.8, 3.9 µg/ml

Incubation Time
GreenScreen HC™: 48 hour exposure time with results collected at 24 and 48 hour time points.

GreenScreen HC S9™: 3 hour exposure in the presence of S9, followed by 45 hour recovery and response time. Measurement at 48 hour timepoint.

Quality Controls
1 % DMSO — Negative vehicle control

GreenScreen HC™: Methyl methanesulfonate (High 50 µg/ml, Low 10 µg/ml) — Positive Control

GreenScreen HC S9™: Cyclophosphamide (High 25 µg/ml, Low 5 µg/ml) — Positive Control

Non-fluorescent control TK6 strain

Test Article Requirements
10 mg solid compound — Other compound preparations are acceptable

Metabolising System
Typically, aroclor-1254 induced rat liver S9

Analysis Method
GreenScreen HC™ — Spectrophotometric reader

GreenScreen HC S9™ — Flow cytometer

Data Delivery
Written report presenting overall results

Lowest effective concentration (LEC) for positive genotoxicity and cytotoxicity results

Excel worksheets with full graphical dose response data for genotoxicity and cytotoxicity

To find out more contact **enquiries@cyprotex.com**

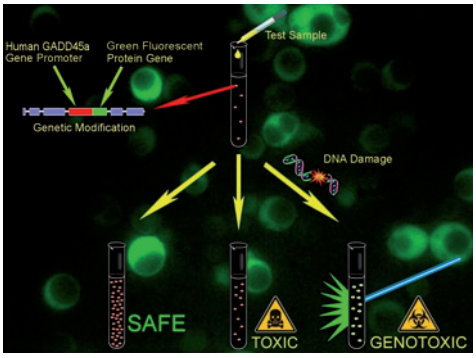
Due to the high throughput, low resource and compound requirement of this assay, *GADD45a*-GFP data can be generated at an earlier stage of the drug development process than is possible with other screening genotoxicity assays, allowing the prioritization of compounds to be progressed into non-clinical studies to support clinical investigations[3].

GreenScreen HC™ Genotoxicity Assessment

GreenScreen HC™ has been validated extensively against a wide range of different *in vitro* and *in vivo* genotoxicity methods.
Results are published within the literature[1,3,4,5]

Figure 1

Schematic diagram illustrating the basic principles of the GreenScreen HC™ assay.



GreenScreen HC™ is a reporter assay which consists of a stably replicating plasmid including all the *cis*-acting regulatory elements of the human *GADD45a* gene, coupled to a gene encoding green fluorescent protein (GFP). GADD45a has a central role in genomic integrity, and genotoxic stress induces its transcription. The reporter system exploits p53-dependent, genotoxin-specific induction of human *GADD45*a expression and the up-regulation leads to production of GFP which is monitored by fluorescent detection (plate reader or flow cytometer). Cytotoxicity is also monitored by optical absorbance, proportional to cell proliferation, in the GreenScreen HC™ assay, and by uptake of propidium iodide dye, proportional to cell viability, in the GreenScreen HC S9™ assay.

Table 1

Predictivity statistics for genotoxic carcinogenicity in a collection of 75 marketed pharmaceuticals[3].

Test	Positive (sensitivity)		Negative (sensitivity)		Concordance	
	n	%	n	%	n	%
GreenScreen HC™	11/12	92	44/47	94	55/59	93
Bacterial mutation	4/12	33	41/45	91	45/57	79
In vitro cytogenetic	12/12	100	21/37	57	33/49	67
In vitro mammalian mutation	5/9	56	30/38	79	35/47	74
In vivo genotoxicity	12/12	100	40/45	89	52/57	91

GreenScreen HC™ exhibits both high sensitivity and specificity for genotoxic carcinogens. If combining the figures for sensitivity and selectivity to generate concordance values, GreenScreen HC™ is found to be the best predictor of genotoxic carcinogens when compared with other methods for assessing *in vitro* and *in vivo* genotoxicity[3].

References

1 Birrell L *et al*, (2010) *Mutation Research* **695**; 87-95
2 Jagger C *et al*, (2009) *Mutagenesis* **24(1)**; 35-50
3 Hastwell PW *et al*, (2009) *Mutagenesis* **24(5)**; 455-463
4 Knight AW *et al*, (2009) *Regul Toxicol Pharmacol* **55**; 188-199
5 Knight AW *et al*, (2009) *J Biomol Screen* **14**; 16-30