

Report Title: devTOX^{qP}-iPSC Assay Results Example

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Stemina Study Number: NA

Sponsor Study Number: NA

Date Test Articles Received: NA

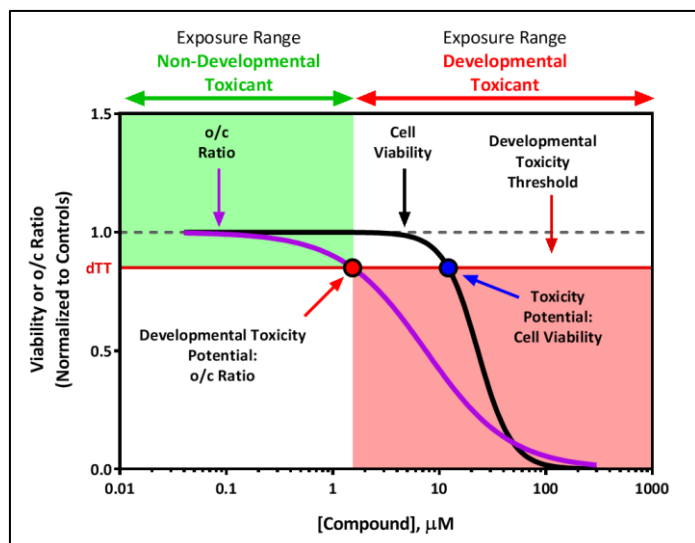
Final Report Date: 14 February 2019

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Sponsor: Name, Inc.

Stemina Project Manager: Jessica Palmer

Stemina's devTOX *quickPredict* is a human pluripotent stem (hPS) cell-based assay that predicts a test article's developmental toxicity potential. The assay uses the metabolic perturbation of the biomarkers ornithine and cystine in a ratio (o/c ratio) to predict the concentration at which a test article shows developmental toxicity potential (dTP). Additional information on the assay methods is provided in Appendix 4 and can be found in detail in Palmer et al. (2013). The current study was conducted using human induced pluripotent stem (iPS) cells.



Interpretation of devTOX^{qP} Results

The dose-response curves for the o/c ratio and cell viability are illustrated with purple and black lines, respectively. The concentration predicted by the point where the dose-response curve of the o/c ratio crosses the developmental toxicity threshold (red line) indicates the exposure level where a metabolic perturbation has developmental toxicity potential (i.e., developmental toxicity potential: o/c Ratio, red point). The toxicity potential concentration from cell viability (blue point) is the point where the cell viability dose-response curve exceeds the developmental toxicity threshold. The developmental toxicity threshold creates a two-sided toxicity model based on exposure: one where exposure does not perturb metabolism in a manner associated with developmental toxicity (green box) and another where exposure shifts metabolism in manner associated with developmental toxicity (red box).

Results Summary

Prediction of the potential for developmental toxicity was made for four test articles through application of the hPS cell-based devTOX *quickPredict* assay. All test articles were blinded to Stemina through use of sponsor-assigned codes. Exposure spanned a range of eight treatment levels per test article (Table 1).

There were three types of responses to test article observed in the data:

- No response in the biomarker ratio and cell viability (N=1).
- A response in the biomarker ratio in the absence of or prior to cell viability response (N=2).
- A response for the biomarker ratio and cell viability at a similar concentration (dTP and TP values are within 3-fold of each other, N=1).

At the exposure levels tested, one test article was in category A (Figure 1) and showed no response for the o/c ratio and cell viability. Based on these data, these test articles are predicted to have little to no potential for developmental toxicity *in vivo* at the exposures tested.

Two test articles were in category B (Figures 2-3), eliciting a metabolic response indicative of the potential for developmental toxicity independent of changes in cell viability. This indicates that these test articles have potential to cause developmental toxicity *in vivo* at or above the predicted dTP concentration.

There was one test article in response category C (Figure 4), causing metabolic perturbation at concentrations within 3-fold of those that impacted cell viability. These test articles are also expected to have the potential for developmental toxicity and/or embryo lethality *in vivo* at or above the dTP concentration.

Table 1: Test Articles Showing a Response in the o/c Ratio Independent of a Cell Viability Response				
Stemina Code	Sponsor ID	Exposure Range Tested (µM)	o/c Ratio dTP (µM)	Cell Viability TP (µM)
Test Articles Showing No Response in the o/c Ratio and Cell Viability				
TPM#EX1	EX#1	0.1-300	ND	ND
Test Articles Showing a Response in the o/c Ratio Independent of a Cell Viability Response				
TPM#EX2	EX#2	0.03-100	0.5	11.2
TPM#EX3	EX#3	0.1-300	23.7	ND
Test Articles Showing a Response in the o/c Ratio and Cell Viability at Similar Concentrations				
TPM#EX3	EX#3	0.1-300	6.7	11.1

dTP: Developmental Toxicity Potential. **TP:** Toxicity Potential. **ND:** No effect detected within the exposure range tested.

o/c Ratio plots for individual test articles are shown in Figures 1-4 (ordered by response category and potency). Included appendices contain information related to individual metabolite response curves for each test article (Appendix 1), performance of the experimental controls (Appendix 2), and test article solubility (Appendix 3).

Response Category A: No Response in the o/c Ratio and Cell Viability

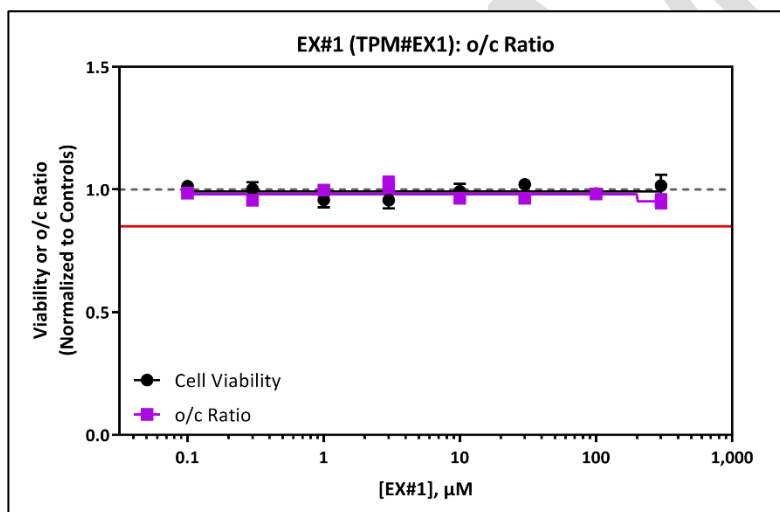


Figure 1: devTOX quickPredict Assay Results for EX#1. The horizontal red line represents the developmental toxicity threshold (0.85). The x-axis is the concentration (µM) of the test article. The y-axis is the reference treatment normalized (fold change) values for the o/c ratio and viability. The points are mean values and error bars are the standard error of the mean. If not shown, error bars are smaller than the size of the symbol.

Response Category B: Response in the o/c Ratio Independent of a Cell Viability Response

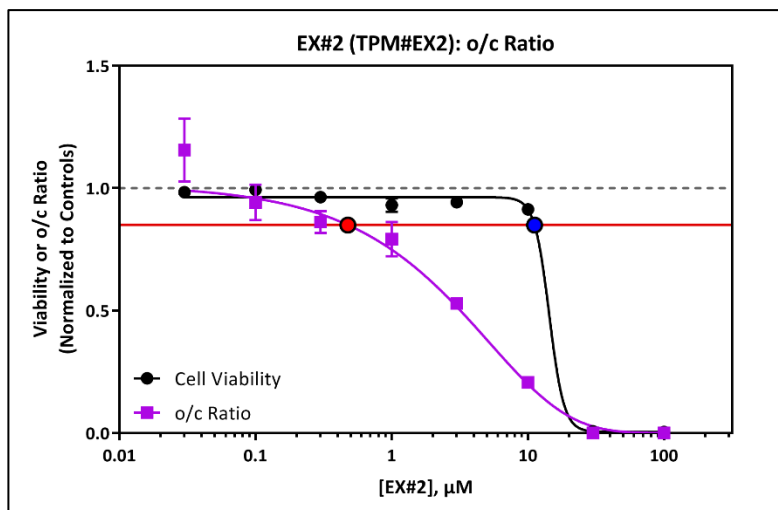


Figure 2: *devTOX quickPredict Assay Results for EX#2.* The horizontal red line represents the developmental toxicity threshold (0.85), the red and blue filled circles indicate the predicted dTP and TP concentrations, respectively. The x-axis is the concentration (μM) of the test article. The y-axis is the reference treatment normalized (fold change) values for the o/c ratio and viability. The points are mean values and error bars are the standard error of the mean. If not shown, error bars are smaller than the size of the symbol.

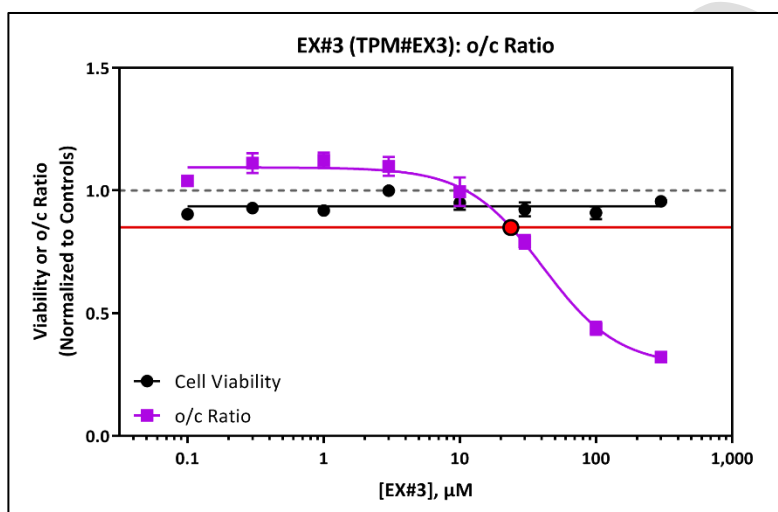


Figure 3: *devTOX quickPredict Assay Results for EX#3.* The horizontal red line represents the developmental toxicity threshold (0.85), the red filled circle indicates the predicted dTP concentration. The x-axis is the concentration (μM) of the test article. The y-axis is the reference treatment normalized (fold change) values for the o/c ratio and viability. The points are mean values and error bars are the standard error of the mean. If not shown, error bars are smaller than the size of the symbol.

Response Category C: Response in the o/c Ratio and Cell Viability at Similar Concentrations

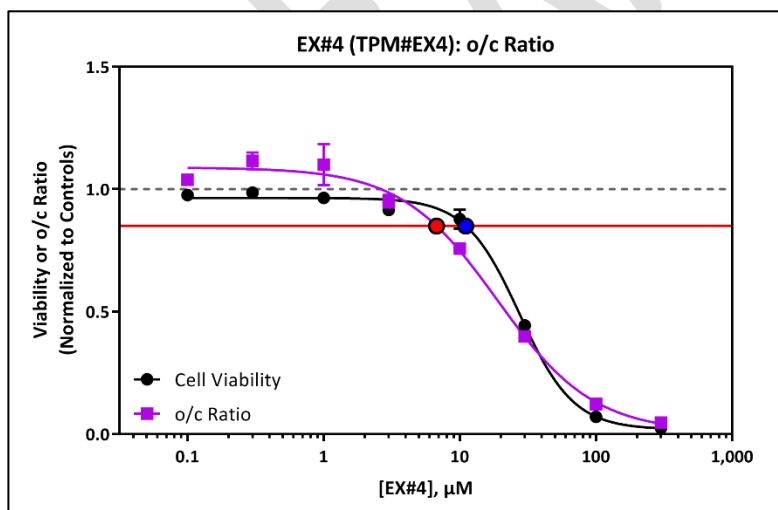


Figure 4: *devTOX quickPredict Assay Results for EX#4.* The horizontal red line represents the developmental toxicity threshold (0.85), the red and blue filled circles indicate the predicted dTP and TP concentrations, respectively. The x-axis is the concentration (μM) of the test article. The y-axis is the reference treatment normalized (fold change) values for the o/c ratio and viability. The points are mean values and error bars are the standard error of the mean. If not shown, error bars are smaller than the size of the symbol.

Appendix 1: Change in Ornithine and Cystine Metabolism Following Test Article Exposure (Ordered by Response Category and Potency)

Response Category A: No Response in the o/c Ratio and Cell Viability

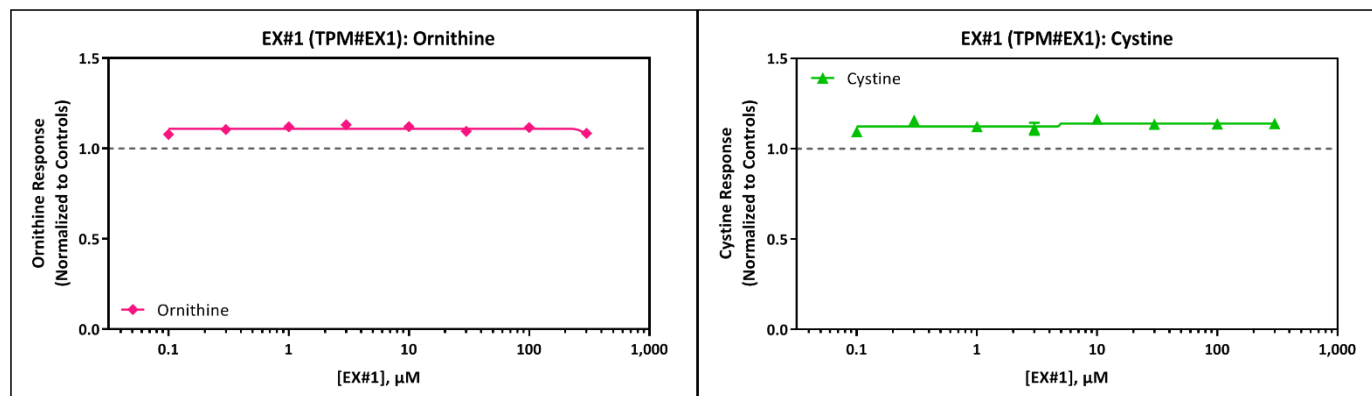


Figure A1.1: Change in Ornithine and Cystine Metabolism Following Exposure to EX#1. The x-axis is the concentration (μM) of the test article. The y-axis is the reference treatment normalized (fold change) values for ornithine or cystine. The points are mean values and error bars are the standard error of the mean. If not shown, error bars are smaller than the size of the symbol.

Response Category B: Response in the o/c Ratio Independent of a Cell Viability Response

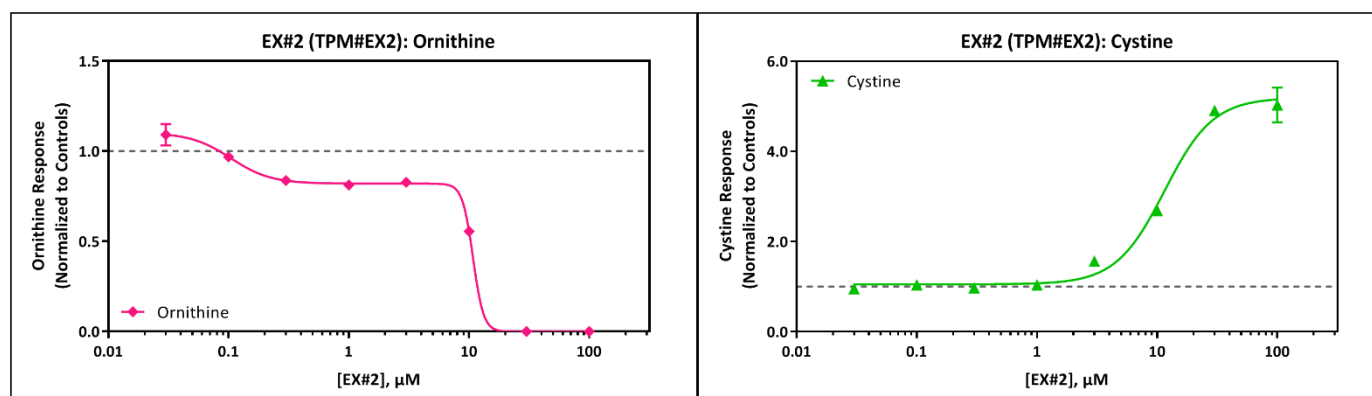


Figure A1.2: Change in Ornithine and Cystine Metabolism Following Exposure to EX#2. The x-axis is the concentration (μM) of the test article. The y-axis is the reference treatment normalized (fold change) values for ornithine or cystine. The points are mean values and error bars are the standard error of the mean. If not shown, error bars are smaller than the size of the symbol.

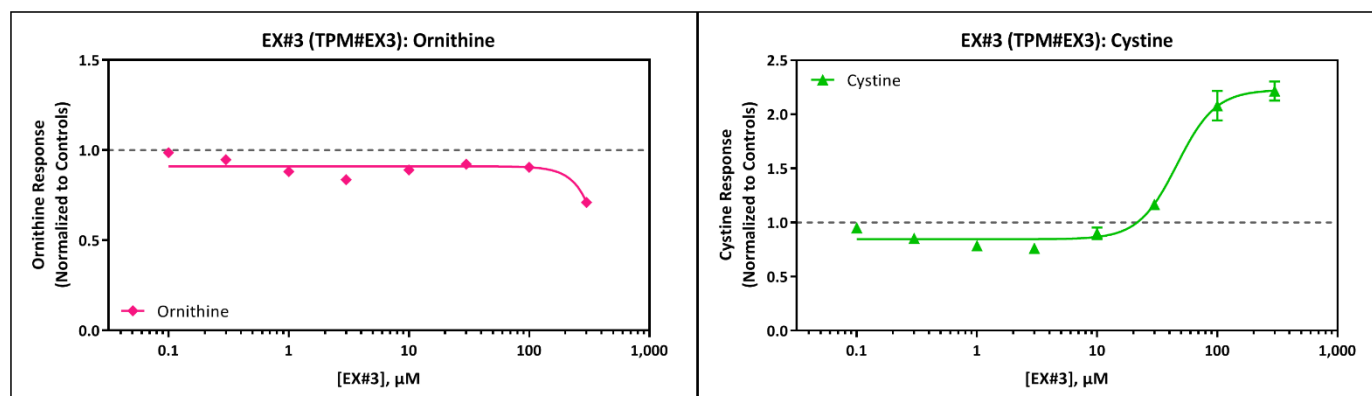


Figure A1.3: Change in Ornithine and Cystine Metabolism Following Exposure to EX#3. The x-axis is the concentration (μM) of the test article. The y-axis is the reference treatment normalized (fold change) values for ornithine or cystine. The points are mean values and error bars are the standard error of the mean. If not shown, error bars are smaller than the size of the symbol.

Response Category C: Response in the o/c Ratio and Cell Viability at Similar Concentrations

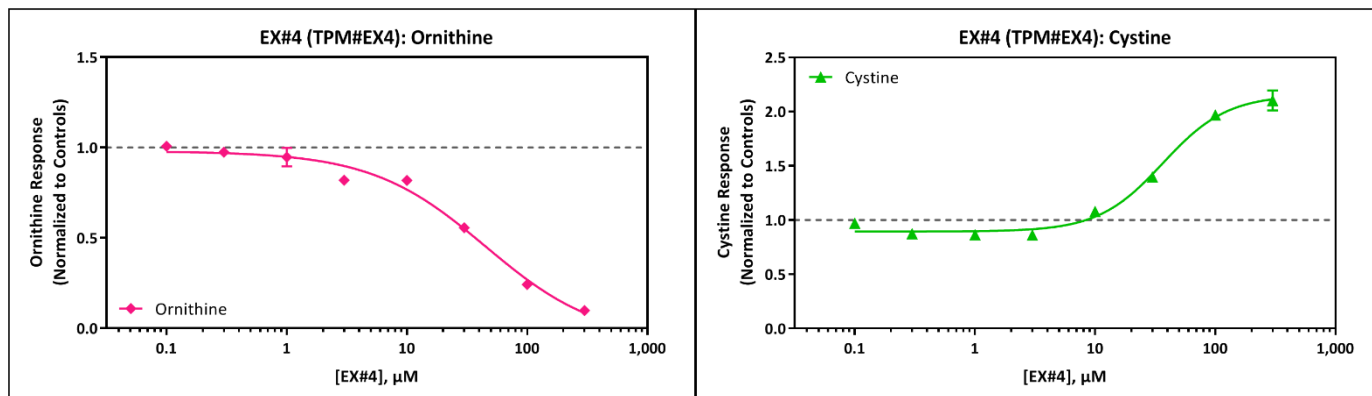


Figure A1.4: Change in Ornithine and Cystine Metabolism Following Exposure to EX#4. The x-axis is the concentration (μM) of the test article. The y-axis is the reference treatment normalized (fold change) values for ornithine or cystine. The points are mean values and error bars are the standard error of the mean. If not shown, error bars are smaller than the size of the symbol.

Appendix 2: Positive and Negative Control Plot Showing the o/c Ratio Predicted Methotrexate Response as Expected

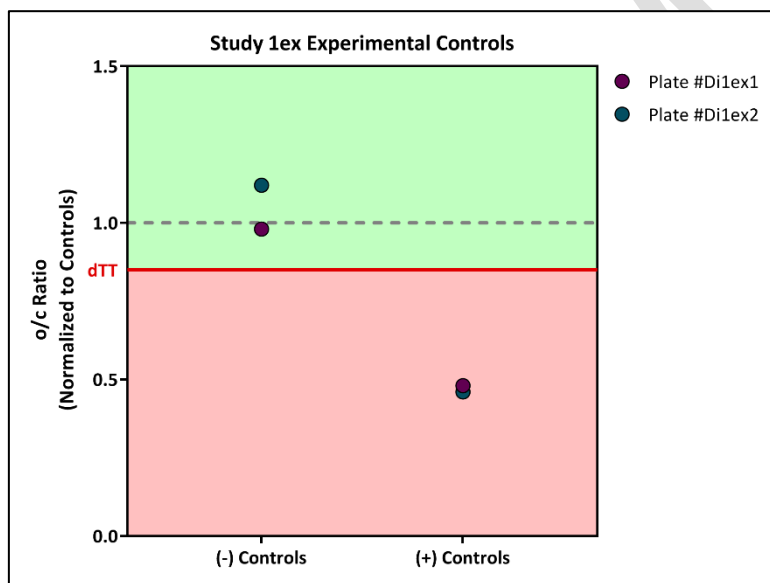


Figure A2.1: Biomarker Ratio Results for Negative and Positive Controls on Each Plate. Controls were included on each cell culture plate and consisted of cells treated with methotrexate at concentrations of 0.005 μM (negative control) and 1 μM (positive control).

Table A2.1: o/c Ratio Response for the Positive and Negative Controls

Control	Treatment	o/c Ratio (±SEM) ¹
Negative	0.005 μM Methotrexate	1.05 (±0.07)
Positive	1.0 μM Methotrexate	0.47 (±0.01)

¹Average o/c Ratio value for 2 experiment plates.

Appendix 3: Test Article Solubility

Initial stock solutions were prepared in 100% DMSO at 300mM. The dosing solutions for the highest exposure level were prepared by taking an appropriate volume of the stock solution and diluting 1:1000 into the mTeSR1 media. If the test article was not soluble in mTeSR1 at 300 μM (based on visual inspection), subsequent dilutions

were performed to determine the maximum concentration at which the test article was soluble in mTeSR1. The exposure range for each test article was based on solubility in mTeSR1 determined by visual inspection during solubility testing.

Table A3.1: Test Article Solubility in DMSO and mTeSR1

Stemina Code	Sponsor ID	Physical State	Storage	Formula Weight (g/mol)	Purity (%)	Density (g/cm ³)	Exposure Range Tested (µM)	DMSO Concentration (mM)	DMSO Solubility	mTeSR1 Concentration (µM)	mTeSR1 Solubility
TPM#EX1	EX#1	Solid	RT	354.32	99.8	N/A	0.1-300	300	Soluble	300	Soluble
TPM#EX2	EX#2	Solid	RT	288.19	96.7	N/A	0.03-100 ¹	300	Soluble	300 100	Not Soluble ² Soluble ³
TPM#EX3	EX#3	Solid	RT	310.24	98.8	N/A	0.1-300	300	Soluble	300	Soluble
TPM#EX4	EX#4	Liquid	RT	406.27	99.9	1.05	0.1-300	300	Soluble	300	Soluble

¹ Exposure range decreased from proposed range due to solubility issues. ² Not Soluble after >15 minutes of sonication. ³ Soluble after ~15 minutes of sonication.

Reference:

Palmer JA, Smith AM, Egnash LA, Conard KR, West PR, Burrier RE, Donley EL, Kirchner FR. Establishment and assessment of a new human embryonic stem cell-based biomarker assay for developmental toxicity screening. Birth Defects Res B Dev Reprod Toxicol. 2013;98(4):343-363.

Signatures:

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