

MultiFlow[®]

DNA Damage Kit – p53, γ H2AX,
Phospho-Histone H3

App Note 3:

Data Analytics and Reporting

*Put your data in the
hands of the experts*



Introduction

MultiFlow App Notes 1 and 2 describe assay installation and the building of a data set for the creation of Machine Learning models. These models allow you to perform your own analyses and interpretation of data. We recognize that not all labs want to take on the entire process from start to finish. You may wish to focus your energy on conducting the assay and not worry about managing the data, interpreting results, or generating reports. Our solution is to provide data quality control and analytics services – essentially putting your data in the hands of the experts.

Quality Control

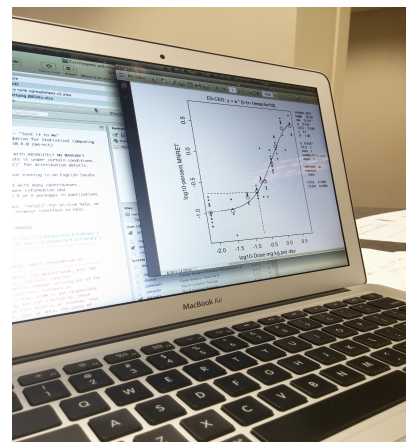
This process is designed to ensure reliable and accurate predictions of genotoxicity and Mode of Action. After installing the assay (App Note 1) and analyzing the Training and Test Sets (App Note 2) you can submit subsequent data to Litron for review of quality. We use a comprehensive checklist to identify potential Quality Control issues.

Using the specialized bead solution included in the kit, instrument settings determined during installation, and Litron's standard 96 well plate format, Litron's Quality Control experts will review:

- Flow cytometer setup values and their effect on bead fluorescence
- Consistency of bead counts and fluorescence across the plate
- Gate positions
- Stability of concurrent negative controls
- Performance of on-plate positive controls
- Other metrics

Data Analytics

Once the entire plate and individual wells are cleared for further processing, results are evaluated against Machine Learning models and Global Evaluations Factors (GEFs). Other than overly cytotoxic conditions ($\geq 80\%$), each concentration is analyzed with all three models (currently Random Forest, Artificial Neural Network, and Logistic Regression).



MultiFlow App Note 3: Data Analytics and Reporting

Results of MultiFlow Study

This plate passed Quality Control (see Appendix 1).

COLOR KEY: Clastogen Aneugen Mixed MOA

Test Article Name	Machine Learning Model Predictions <small>(for details, see Predictions from ML Models tab)</small>			Global Evaluation Factor Predictions <small>(for details, see Predictions from GEF tab)</small>			Final Predictions		Notes
	Evidence of Genotoxicity?	Genotoxic Mode of Action	Lowest Observed Effect Concentration	Evidence of Genotoxicity?	Genotoxic Mode of Action	Lowest Observed Effect Concentration	Evidence of Genotoxicity?	Genotoxic Mode of Action	
Etoposide	Yes	Clastogen	0.062424534 µM	Yes	Clastogen	0.124886784 µM	Yes	Clastogen	
Mebendazole	Yes	Aneugen	0.055117715 µM	Yes	Aneugen	0.110268732 µM	Yes	Aneugen	
Hexachloroethane	No	N/A	N/A	No	N/A	N/A	No	N/A	

The spider graphs below show the fold increases (above solvent control average) for the following Global Evaluation Factors: Pan-genotoxic GEF 24Hr p53, clastogenic GEFs 4Hr H2AX, 24Hr H2AX, 4Hr p53, 24Hr ph-H3, and aneugenic GEFs 4Hr ph-H3, and (sqrt of) 24Hr polyploidy.

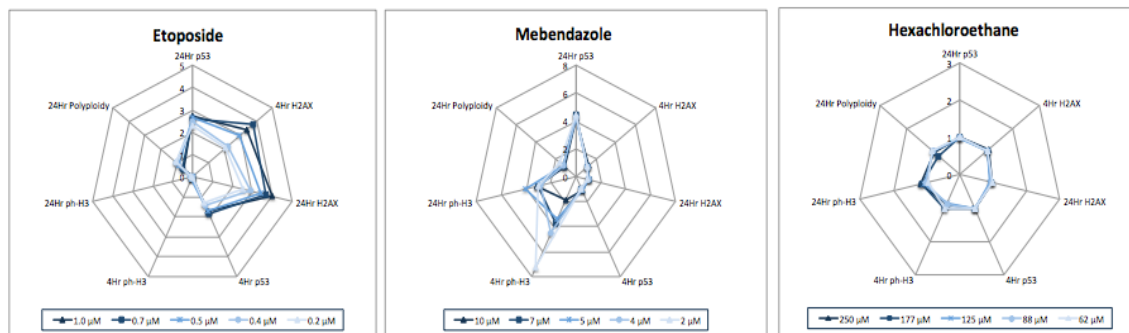


Figure 1: Example output from Litron report. The table shows predictions made by Machine Learning models and GEFs. The spider plots summarize activity of compounds across multiple endpoints.

The results of these Machine Learning models are then combined using an ensemble approach to provide a single prediction of clastogenicity, aneugenicity or nongenotoxicity for each test article. Each concentration is also compared to GEFs, and in a similar manner, a single prediction of clastogenicity, aneugenicity or nongenotoxicity is made for each test article.

Reporting

It can take a considerable effort to convert complex results from GEFs and Machine Learning models into an easy-to-understand format. Litron has built custom spreadsheets that provide both a high-level summary along with data for individual wells. Results that fail to achieve specified QC criteria are flagged to help troubleshoot and resolve issues for future analyses. Predictions are made for overall genotoxicity as well as Mode of Action. Responses for individual endpoints are displayed as spider plots for each test article (Figure 1).

Conclusions

This App Note describes the Quality Control and Data Analysis process available from Litron. This flexibility is just another aspect of what makes choosing MultiFlow the best decision you can make for your genotoxicity profiling requirements.

Full Service Option

If you'd like to have the benefits of MultiFlow but aren't ready to perform the method in-house, we now offer a full service option. Send your test articles to the experts at Litron. We will conduct the entire experiment from exposure (currently TK6 cells only) to analysis and report generation. Many groups use this option to understand the performance of MultiFlow for their chemical space prior to adopting it in-house. Talk to us today about your needs and let us know how we can help.