Toxicology Testing

Genetic Toxicology Service List

Basic Chemicals

BioReliance® by SAFC

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Regulatory Battery

BioReliance is a leading provider of services for Genetic Toxicology evaluation to meet global regulatory guidelines. The tables below list the categories and assays required for different regulations.

Governing Agency	Guideline	Harmonized Protocols	Reference	GLP	Legislation/ Regulation
US Environmental Protection Agency (EPA)	OCSPP	OECD	**www.epa.gov/epahome/lawregs.htm**	EPA 40 CFR Part 792 (TSCA)	Toxic Substances Control Act (TSCA)
European Chemicals Agency (ECHA)	REACH	OECD	**www.echa.europa.edu**	Directives 2004/9/EC and 2004/10/EC	Council Regulation (EC) No 1907/2006
Ministry of Economy, Trade and Industry (METI)	CSCL	OECD	**www.meti.go.jp**	Act No. 39	Act on the Evaluation of Chemical Substances and Regulation of Their Manufacture (Act No. 117)

Regulatory Office	Guidelines	Assay Group	Assays
US EPA – Office of Pollution Prevention and Toxics (OPPT)	OCSPP (Office of Chemical Safety and Pollution Prevention) Harmoniized Test Guidelines	Series 870 – Health Effects Test Guidelines	Group D – Genetic Toxicity Test Guidelines 870.5100 – Bacterial Reverse Mutation Test 870.5300 – In vitro Mammalian Cell Gene Mutation test 870.5375 – In vitro Mammalian Chromosome Aberration Test 870.5380 – Mammalian bone Marrow Chromosomal Aberration Test 870.5395 – Mammlian Erythrocyte Micronucleus Test 870.5550 – Unscheduled DNA Synthesis in Mammalian Cells in Culture 870.5900 – In vitro Sister Chromatid Exchange Assay 870.5915 – In vivo Sister Chromatid Exchange Assay
ECHA	REACH	Annexes VII, VII, IX, X	See Below
METI	CSCL	Toxicity tests	OECD TG471 – Bacterial reverse Mutation Test OECD TG473 – In vitro Mammalian Chromosome Aberration Test OECD TG407 – Repeated Dose 28-day Oral Toxicity Study in Rodents

Regulatory Assay Options (REACH)

Class of Chemical (tonnes)	REACH Annex	REACH Section	Assay			OECD Guideline	REACH Test Method	Rules for Adaptation
			Category	Description	Name			
1 - 10	VII	8.4	Mutagenicity	In vitro gene mutation study in bacteria	Ames Assay	471	B13/14	If positive further studies considered (Annex VIII)
10 - 100	VIII	8.4	Mutagenicity	In vitro cytogenicity study in mammalian cells or in vitro micronucleus study	In Vitro Chromosome Aberration Assay	473	B10	If positive further studies considered (Annex IX, X)
					In Vitro Micronucleus Assay	487	B12	
				In vitro gene mutation study in mammalian cells	CHO/HPRT assay or Mouse Lymphoma Assay	476	B17	
100 - 1000	IX	8.4	Mutagenicity	in vivo somatic cell genotoxicity study	In Vivo Chromosome Aberration Assay	475	B11	If positive consider germ cell mutagenicity
					In Vivo Micronucleus Assay	474	B12	
> 1000	X			2nd in vivo somatic cell genotoxicity study	see above (Annex IX assays)			If positive consider germ cell mutagenicity

Other In vivo assays to follow up in vitro positive

In vivo UDS Assay	486	B39
Big Blue Transgenic Rodent Mutation Assay	488	

For Specific Protocols, see page 14

GLP In Vitro Assays

Bacterial Mutation Assays (Ames)

OECD 471 - *Salmonella / Escherichia coli* Reverse Mutation Assay

This assay detects reverse mutations at histidine genes in *Salmonella typhimurium* or a tryptophan gene in *Escherichia coli*. The bacterial reverse mutation assay, commonly called the Ames assay, typically employs five tester strains (for example, *Salmonella* TA98, TA100, TA1535, and TA1537 and *E. coli* WP2 *uvr*A) each tested with and without S9 activation. Additional or alternate tester strains may be used as described in the OECD guidelines. Any version of the Ames Assay can be performed with any or all of the strains specified by OECD.

Regulatory Guidance on Independent Repeat Assays: It is not necessary to conduct an independent repeat assay when the test article is clearly positive or when it is negative (justification is required for OECD, but not ICH). Per both the ICH and OECD guidelines, equivocal results require retesting using an appropriate modification of the experimental design (e.g., dose levels, activation system or treatment method). The inclusion of two independent mutation data sets is incompliance with OECD.

Study Phases: Each phase is conducted with the selected tester strains both with and without S9 activation. A preliminary toxicity assay consists of a minimum of eight test article dose levels (one plate per dose) and concurrent vehicle controls. A toxicity-mutation assay consists of a minimum of eight test article dose levels (two replicate plates per dose) and concurrent vehicle and positive controls. A mutation assay consists of a minimum of five test article dose levels (three replicate plates per dose) and concurrent vehicle and positive controls.

Exposure Methods: The plate incorporation method combines the test article and test system in top agar just prior to plating; whereas the preincubation method co-incubates the test article with the test system before mixing with top agar and plating. The preincubation method is considered the more sensitive method for the detection of mutagens, especially for certain classes of chemicals.

Protocol*	Assay Description
502	**ICH/OECD protocol - Plate incorporation method:** Preliminary toxicity assay and one mutation assay.
502001	**ICH/OECD protocol - Plate incorporation method with independent repeat:** Preliminary toxicity assay and two mutation assays (initial and confirmatory).
502002	**ICH/OECD protocol - Plate incorporation method with preincubation independent repeat:** Plate incorporation method for preliminary toxicity assay and initial mutation assay. Preincubation method for independent repeat assay.
502005	**OECD protocol - Plate incorporation method:** No preliminary toxicity assay, one mutation assay.
503	**OECD protocol - Plate incorporation method with two trials:** One toxicity-mutation assay and a confirmatory mutation assay.
503001	**OECD protocol – Preincubation method with two trials:** One toxicity-mutation assay and a confirmatory mutation assay.
503005	**OECD protocol - Plate incorporation/preincubation with two trials:** One toxicity-mutation assay (plate incorporation) and a confirmatory mutation assay (preincubation).
504	**OECD protocol – Preincubation method:** Preliminary toxicity assay and one mutation assay.
504001	**ICH/OECD protocol – Preincubation method with independent repeat:** Preliminary toxicity assay and two mutation assays.
507001	**ICH/OECD protocol – Treat and Plate Method with two trials:** One toxicity-mutation assay and a repeat mutation assay. **(**for compounds containing histidine or tryptophan)

***Specialized protocols for reductive conditions, vapor or gas-phase exposure, and nonstandard S9 activation systems are available upon request.**

In Vitro Mammalian Cell Gene Mutation Assays

OECD 476 - L5178Y TK$^{+/-}$ Mouse Lymphoma Assay

This assay measures induction of mutations at the thymidine kinase (TK) gene in L5178Y mouse lymphoma cells. Preliminary toxicity assay and mutation assay performed with (+S9) and without (-S9) metabolic activation. Mutation assay includes cloning five to eight test article concentrations depending on protocol; three replicate plates per dose for cytotoxicity and mutant selection; concurrent vehicle and positive controls; colony sizing provided for vehicle and positive controls and for mutagenic test articles. For protocol 704, an additional non-activation assay with 24 hours continuous exposure is performed if initial 4 hour non-activation exposure is negative. All protocols use soft agar for mutant selection.

Protocol	Assay Description
704	**ICH/OECD protocol - Duplicate cultures per dose level:** Treatments of 4 and 24 hours without S9 and 4 hours with S9.
700	**OECD protocol - Single culture per dose level** Treatment of 4 hours with and without S9.
702	**OECD protocol - Duplicate cultures per dose level** Treatment of 4 hours with and without S9.
702001	**OECD protocol - Duplicate cultures per dose level with independent repeat** Treatment of 4 hours with and without S9.

OECD 476 - CHO/HPRT Gene Mutation Assay

This assay measures induction of mutations at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) gene in Chinese hamster (CHO) cells. Preliminary dose-finding study and mutation assay performed with and without S9 activation. Includes: cloning five test article concentrations; triplicate plates per replicate culture for cytotoxicity and five plates per replicate culture for mutant selection; concurrent vehicle and positive controls. *Other cell lines available, including V79 (please inquire).*

Protocol	Assay Description
782	**OECD protocol - Duplicate cultures per dose level.** Treatment of 5 hours with and without S9.
782001	**OECD protocol - Duplicate cultures per dose level with independent repeat.** Treatment of 5 hours with and without S9.

In Vitro Cytogenetics

OECD 473 - Chromosomal Aberration Assays

This protocol is designed per OECD test guideline 473*. This assay measures chromosomal aberrations and polyploidy in vitro in: human peripheral blood lymphocytes (HPBL), Chinese hamster ovary (CHO) or Chinese hamster lung (CHL) cells and Rat Lymphocytes. Studies performed with (+S9) and without (-S9) metabolic activation, and includes: mitotic inhibition or cell growth inhibition (utilizing relative increase in cell counts) as a measure of toxicity, depending on source of cells. 150 metaphases are scored per culture, 300 per dose level.
* Revised OECD guidelines adopted 9/26/14 allow for new and old versions to be employed for a period of 18 months. Designs listed here reflect new requirements. Old designs are available upon request.

Chromosomal Aberrations in Human Peripheral Blood Lymphocytes

Protocol	Assay Description
341	**ICH/OECD protocol (HPBL) -** Treatments of 4 and 20 hours without S9 and 4 hours with S9; cells collected at 20 hours. Numerical and structural aberrations analyzed in a minimum of 3 dose levels in duplicate. Includes mitotic inhibition as a measure of toxicity
331	**ICH/OECD protocol (CHO or CHL) -** Treatments of 4 and 20 hours without S9 and 4 hours with S9; cells collected at 20 hours. Numerical and structural aberrations analyzed in a minimum of 3 dose levels in duplicate. Includes cell growth inhibition, utilizing relative increase in cell counts (RICC) as a primary measure of toxicity and mitotic index as a secondary measure of toxicity.

Chromosomal Aberrations in Rat Lymphocytes

Protocol	Assay Description
140R	**Chromosome Aberrations in Rat Lymphocytes:** In vitro treatments of 4 and 24 hours without S9 and 4 hours with S9; cells collected at 24 hours. Minimum of three dose levels in duplicate with concurrent vehicle and positive controls. Includes mitotic index as a primary measure of toxicity.

OECD 487 - In Vitro Micronucleus Assay

This protocol is designed per OECD test guideline 487*. The assay measures micronucleus formation in vitro, using human TK6 Cells, Chinese hamster ovary (CHO) cells, or human peripheral blood lymphocytes. A specialty assay in EpiDerm™ 3D reconstructed human skin model is also available. Studies are performed with (+S9) and without (-S9) metabolic activation. Studies scored microscopically include an evaluation of the frequency of binucleated cells as a measure of toxicity. Micronucleus studies in CHO cells can be scored using flow cytometry.
* Revised OECD guidelines adopted 9/26/14 allow for new and old versions to be employed for a period of 18 months. Designs listed here reflect new requirements. Old designs are available upon request.

Protocol	Assay Description
361	**In Vitro Micronucleus Assay in TK6 Cells:** Treatments of 3-6 and 27 hours without S9 and 3-6 hours with S9, 9 doses for toxicity assessment, up to 4 dose levels evaluated for MN induction, 4000 cells scored per dose level.
348	**In Vitro Micronucleus Assay in HPBL:** Treatments of 4 and 24 hours without S9 and 4 hours with S9, 9 doses for toxicity assessment, minimum of three dose levels evaluated for MN induction, 4000 cells scored per dose level.
368	**In Vitro Micronucleus Assay in CHO Cells:** In situ method, treatments of 4 and 24 hours without S9 and 4 hours with S9, 9 doses for toxicity assessment, minimum of three dose levels evaluated for MN induction, and 4000 cells scored per dose level. Scoring by microscopy, with option for flow cytometric evaluation (please inquire).
358	**Reconstructed Skin Micronucleus Assay:** Treatments of up to 6 dose levels in triplicate (score 3).

Options for Mechanistic Analysis in Cytogenetic Assays

FISH and CREST labeling investigate the mode of action for a test article that is positive in a micronucleus assay. FISH and CREST labeling identify whether a micronuclei contains a whole chromosome(s), as a result of an aneugenic mechanism, or a chromosome fragment(s) as a result of a clastogenic mechanism. An aneugenic mechanism may be useful in identifying a threshold, which is very important in the overall risk assessment of a compound.

FISH (Fluorescent in situ Hybridization)

Centromeric labeling of micronuclei by FISH is a cytogenetic technique that is used to detect the presence or absence of centromeric DNA sequences on chromosomes.

Protocol	Assay Description
348FISH	**In Vitro Micronucleus Assay in HPBL with FISH Option Preparation:** Same design as 348, but includes triplicate cultures and slide preparation for FISH analysis.
348FISHselect	**In Vitro Micronucleus Assay in HPBL with FISH Option Preparation in select doses:** Limited treatments and doses as chosen from previous MN assay with positive results. Score all selected doses (triplicate cultures per treatment). Slides prepared for FISH analysis.
FISH_001	**Slide Preparation in HPBL, TK6 or CHO Cells and Analysis:** Slides labeled (stained) for up to 3 treatment conditions. Evaluation of dose level statistically positive for the presence of micronuclei.

* FISH Evaluation can be added to 361 (MN in TK6 cells) without additional preparation (FISH_001 only)

CREST Labeling

CREST uses a human autoimmune antibody that binds to kinetochores of chromosomes across species.

Protocol	Assay Description
348CREST	**In Vitro Micronucleus Assay in Isolated HPBL with CREST Option Preparation:** Similar design as 348, but includes additional culture and slide preparation for CREST option.
348CRESTselect	**In Vitro Micronucleus Assay in HPBL with CREST Option Preparation in select doses:** Limited treatments and doses as chosen from previous MN assay with positive results. Score all selected doses (triplicate cultures per treatment). Slides prepared for CREST option.
368CREST	**In Vitro Micronucleus Assay in CHO Cells with CREST Option Preparation:** Same design as 368, but includes additional culture and slide preparation for CREST option.
368CRESTselect	**In Vitro Micronucleus Assay in CHO Cells with CREST Option Preparation in select doses:** Limited treatments and doses as chosen from previous MN assay with positive results. Score all selected doses, Slides prepared for CREST option.
CREST_004	**In Vitro Micronucleus Option – Anti-Kinetochore Labeling:** Slides stained (from 348) for up to 3 treatment conditions and PCs. Evaluation of dose level(s) statistically positive for the presence of micronuclei.
CREST_001	**In Vitro Micronucleus Option – Anti-Kinetochore Labeling:** Slides stained (from 368) for up to 3 treatment conditions and PCs. Evaluation of dose level(s) statistically positive for the presence of micronuclei.

* CREST Evaluation can be added to 361 (MN in TK6 cells) without additional preparation

Slide Scoring Options

Protocol	Assay Description
150	**Slide Scoring of CHO Cells/Human Lymphocytes:** In vitro chromosome aberration scoring of sponsor-provided slides. One hundred-fifty metaphases per slide.

Morphological Transformation

BALB/3T3 Transformation Assay

This assay detects the ability of a test article to change BALB/3T3 cells from the normal contact-inhibited state to a transformed state characterized by the loss of contact inhibition. Cells that undergo transformation grow into colonies with characteristic morphology and growth characteristics. Preliminary toxicity and definitive assay performed with and without S9 activation. Preliminary toxicity assay includes cloning efficiency in triplicate plates for definitive assay dose selection. Definitive assay includes four dose levels, concurrent positive and negative controls, 15-20 plates per dose level for transformation potential, and three plates per dose level for concurrent cytotoxicity.

Protocol	Assay Description
304	**In Vitro Transformation Assay in BALB/3T3 Cells with and without S9 activation**

In Vitro Syrian Hamster Embryo (SHE) Transformation Assay

This assay detects the ability of a test article to induce preneoplastic changes in primary Syrian hamster embryo cells from a normal contact-inhibited growth to morphologically transformed state colonies characterized by the loss of contact inhibition and normal growth patterns. Cells that undergo transformation grow into colonies with characteristic morphology and growth characteristics. Two study design options are available with dose administration of 24 hours or 7 days. Preliminary toxicity assay performed to establish the dose range and to adjust the target cell seeding density for the definitive assay. Definitive assay includes five dose levels, concurrent negative and positive controls, and 40 plates per dose level for transformation potential.

Protocol	Assay Description
308	**In Vitro Transformation of Syrian Hamster Embryo Cells – 7 Day Exposure**
309	**In Vitro Transformation of Syrian Hamster Embryo Cells – 24 Hour Exposure**
310	**In Vitro Transformation of Syrian Hamster Embryo Cells – 7 day and 24 Hour Exposure**
308001	**Syrian Hamster Embryo (SHE) Cells:** SHE cells prepared from mid gestation (13 days) hamster embryos, which are grown for 24 to 48 hours and cryopreserved. These cells are evaluated for plating efficiency (above 20%), and response toBenzo (a) pyrene and negative control. Frozen cells are available for use in the SHE cell transformation assay frozen at 2.5×10^6 (feeder cells) or 1.0×10^6 (target cells).

GLP In Vivo Assays

In Vivo Cytogenetics

These assays detect damage induced by the test article to alter chromosome structure or interfere with the mitotic apparatus causing changes in chromosome number.

Study Designs

Below are many options that fulfill regulatory requirements, depending on sponsor preference and test article specifics. Each assay will require a dose range finding test (unless data is available from the Sponsor) that is listed on the next page.

OECD 474 - In Vivo Micronucleus Assay

The purpose of the micronucleus test is to identify substances that cause cytogenetic damage, which results in the formation of micronuclei containing lagging chromosome fragments or whole chromosomes (an aneugenic or clastogenic mechanism).

Microscopic Scoring: Mice or rats are used to detect micronuclei in polychromatic erythrocytes in the bone marrow or peripheral blood. Typically, three test article dose levels, concurrent vehicle and positive controls, dose administration followed by one, two, or more bone marrow collection time points, five animals/sex/dose level are used. Plasma or serum collection for demonstration of test article bioavailability available with all *in vivo* studies is available at additional cost. Dose selection is based on a dose range finding test. Assay can be conducted with males only if there is no difference in toxicity between the sexes in the dose range finding assay

Flow Cytometric Scoring: Mice or rats are used to detect micronuclei in polychromatic erythrocytes (PCE) in the bone marrow or in reticulocytes (RET) in peripheral blood. Flow cytometric analysis adds the benefits of automated scoring, increased sample size, and improvements in measurement and cytotoxicity indications. Standard protocols are listed below. Other options and assay designs are listed on the next page. Please inquire for recommendations.

* Revised OECD guidelines adopted 9/26/14 allow for new and old versions to be employed for a period of 18 months. Designs listed here reflect new requirements. Old designs are available upon request.

Protocol #	Assay Description
Mice	
123012	**Mouse bone marrow - ICH/OECD protocol (Microscopic Scoring):** Single administration, two collection time points, vehicle controls at each collection time point, 4000 PCE evaluated per animal, 35 mice/sex. Protocol 123M012: males only
123021	**Mouse bone marrow - ICH/OECD protocol (Microscopic Scoring):** Two administrations, one collection time point, 4000 PCE evaluated per animal, 25 mice/sex. Protocol 123M021: males only
Rats	
125012	**Rat bone marrow - ICH/OECD protocol (Microscopic Scoring):** Single administration, two collection times, vehicle controls at each collection time point, 4000 PCE evaluated per animal, 35 rats/sex. Protocol 125M012: males only
125021	**Rat bone marrow - ICH/OECD protocol (Microscopic Scoring):** Two administrations, one collection time point, 4000 PCE evaluated per animal, 25 mice/sex. Protocol 125M021: males only

In Vivo Cytogenetics

Additional Analyses for the In Vivo Micronucleus Assay

Option	**Body temperature monitoring:** Hypothermia and hyperthermia are both known to be associated with production of micronuclei in rodent bone marrow hematopoietic cells, through aneugenesis. A variety of drugs have been shown to raise or lower core body temperature sufficiently to cause micronuclei formation. Implantable temperature transponders may be used to investigate alterations in body temperature

Cytogenetic Analysis Options

As with in vitro assays, FISH and CREST labeling are available to investigate the mode of action for a test article that is positive in an in vivo micronucleus assay.

FISH (Fluorescent in situ Hybridization)

Centromeric labeling of micronuclei by FISH is a cytogenetic technique that is used to detect and localize the presence or absence of specific centromeric DNA sequences on chromosomes.

Protocol	Assay Description
To be used with 123	**Additional Preparation for FISH with In Vivo Micronucleus Assay:** Aneugenic positive control added. Filter bone marrow cells through cellular column. Prepare slides at -30°C for potential FISH.
123FISHselect	**In Vivo Micronucleus Assay in with FISH Option Preparation in select doses:** Definitive assay with select doses. Extra slides prepared for FISH option and stored at -30°.
FISH_002	**In Vivo Micronucleus Option – FISH:** Process slides from cultures selected for FISH analysis. Scoring for centromere positive micronuclei. Evaluate and report FISH data (Mouse Only).

CREST Labeling

CREST uses a human autoimmune antibody that binds to kinetochores of chromosomes across species.

Protocol	Assay Description
To be used with 123 or 125	**Additional Preparation for CREST with In Vivo Micronucleus Assay:** Aneugenic positive control added. Filter bone marrow cells through cellular column. Prepare slides at -30°C for potential CREST.
___CRESTselect	**In Vivo Micronucleus Assay in with CREST Option Preparation in select doses:** Definitive assay with select doses. Extra slides prepared for CREST option and stored at -30°.
CREST_002	**In Vivo Micronucleus Option – Anti-Kinetochore Labeling:** Slides stained. Evaluation pf dose level statistically positive for the presence of micronuclei.

In Vivo Cytogenetics

OECD 475 - Chromosome Aberration Analysis in Rodents

Rodent cytogenetic assays using metaphase analysis measure induction of chromosome aberrations and polyploidy in vivo. Typically, three test article dose levels, concurrent vehicle and positive controls; single dose administration followed by two or more bone marrow collection time points, five animals per sex per dose level for each time point are used. Plasma or serum collection for demonstration of test article bioavailability is available with all in vivo studies at additional cost. Dose selection is based on a dose range finding test. Option for males only is allowed if no difference in toxicity between the sexes is seen in the dose range finding assay.
* Revised OECD guidelines adopted 9/26/14 allow for new and old versions to be employed for a period of 18 months. Designs listed here reflect new requirements. Old designs are available upon request.

Protocol	Assay Description
108	**ICH/OECD protocol - Mouse bone marrow:** Single administration, two collection time points, vehicle controls at each time point, 200 metaphase cells evaluated/animal, 35 mice/sex. Numerical aberrations evaluated at all dose levels. Protocol 108M: males only
126	**Mouse bone marrow assay for determination of clastogenicity/aneuploidy:** Polychromatic erythrocytes evaluated using immunofluorescent techniques to differentiate micronuclei with and without kinetochores.
107GLP	**ICH/OECD protocol - Rat bone marrow:** Single administration, two collection time points, vehicle controls at each time point, 200 metaphase cells evaluated/animal, 35 rats/sex. Numerical aberrations evaluated at all dose levels. Protocol 107M: males only

Dose Range Finding Tests for In Vivo Studies

Each in vivo study requires dose range finding tests to help in the selection of the appropriate dose levels for the definitive study. The dose range finding test includes a modified limit test to determine the maximum tolerated dose (MTD), which will be used as the highest dose in the definitive study, with 2000 mg/kg being the limit dose. The test article will be initially evaluated using 3 male and 3 female in 3 dose groups. In order to further define the MTD, an extensive toxicity test may follow.

Protocol	Assay Description
000M	**Mouse Dose Range Finding Test (Males and females)**
000R	**Rat Dose Range Finding Test (Males and females)**
000RM	**Rat Dose Range Finding Test** (males only)

DNA Damage and Repair

OECD 486 - In Vivo/In Vitro Unscheduled DNA Synthesis (UDS)

This assay detects repair of DNA in vitro in male rat or mouse hepatocytes collected from animals treated in vivo with test article. Three test article dose levels, concurrent positive and negative controls, single dose administration using 5 animals per dose level with 3 animals per dose evaluated for UDS using auto-radiography, two time points. Dose selection based on dose range finding test.

Protocol	Assay Description
381 *ICH S2 Design*	**Rat Liver UDS Assay:** 40 animals dosed, 30 animals evaluated. 3 dose levels, 1 dose administration, 2 harvests.
383	**Mouse Liver UDS Assay:** 40 animals dosed, 30 animals evaluated 3 dose levels, 1 dose administration, 2 harvests
384	**Mouse Liver UDS Assay:** 45 animals dosed, 30 animals evaluated.
000RM	**Rat Dose Range Finding Test** (males only)

In Vivo Gene Mutation

OECD 488 - Big Blue® Transgenic Rodent Mutation Assay

BioReliance's Big Blue® Transgenic Rodent Mutation (TRM) assay utilizes a novel transgenic animal model bred for specific use in an assay defined by OECD Test Guideline 488, "Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays." This assay allows the measurement of mutations in any tissue including germ cells and has been re-qualified for commercial use. For analysis, Mutation frequency is denoted by dividing the number of mutant plaques over total number of plaques.

Protocol	Assay Description
Mice/Rats 170/171	**Plaque Counting with Rodent Tissue:** 5 doses (1 vehicle control, 1 positive control, 3 dose levels). 5 male rodents per group. 2 tissues analyzed, 125,000 phage evaluated per tissue; detect 2-fold increase over background with 80% confidence.

Big Blue® Transgenic Rodents

Big Blue® mice and rats are bred to have multiple copies of recoverable target genes integrated into their genome. Big Blue® animals are created by a microinjection of the lambda shuttle vector containing the *cII* gene into the pronucleus of fertilized eggs from either C57Bl/6 mice or Fisher 344 rats. BioReliance has exclusive license to breed these animals which are available commercially.

Protocol	Assay Description
Mice BBMM_BL6_HOMO	**Big Blue® C57BL/6 Transgenic Homozygous Mouse:** Both males and females available.
Rats BBMR_F344_HOMO	**Big Blue® Fisher 344 Transgenic Homozygous Rat:** Both males and females available..

Genetic Toxicology Endpoints

Many in vivo studies have scoring and evaluation that can be separately added on to the end of the in-life portion of studies. Endpoints can be evaluated by Flow Cytometry, or manual scoring. Entire studies may be conducted within BioReliance's fully accredited animal facility, or added to ongoing animal studies from other facilities. In the case of samples sent to BioReliance from other studies, specific collection procedures and kits are provided. Onsite collection services, for sponsor-run repeat dose animal toxicology studies, are available. Please inquire.

Micronucleus

Protocol	Assay Description
129GLP	**Slide scoring:** Micronucleus evaluation of rodent bone marrow or peripheral blood slides. 2000 cells scored per slide (4000 per animal). Includes staining, evaluation, and summary report.
129FlowPBGLP *ICH S2(R1) Option*	**Flow cytometric scoring:** Micronucleus evaluation of samples from whole blood. 20,000 cells scored per sample. Includes summary report.
129FlowPBFGLP	**Flow cytometric scoring:** Micronucleus evaluation of prepared samples (sponsor-provided fixed blood). 20,000 cells scored per sample. Includes summary report.

Chromosome Aberration

Protocol	Assay Description
149	**Slide Scoring:** Evaluation of sponsor-provided slides from rodent bone marrow or cells. 200 metaphases per slide. Includes staining, microscopic evaluation and summary report.

Big Blue®

Protocol	Assay Description
172	**Plaque Counting:** 125,000 phage evaluated per tissue; detect 2-fold increase over background with 80% confidence.

Analytical Chemistry

Analytical support is an important and required part of any GLP assay. Analytical methods must be available or developed before a GLP assay is performed. In addition, stability analysis and dosing formulation verification are available to support Genetic Toxicology assays and protocols. BioReliance offers fully-validated, GLP-compliant equipment, software, and study designs. Our state-of-the-art equipment includes: LC/MS/MS, HPLC, GC, and UV/Vis.

Analytical Method Validation

These studies are available to validate an analytical method for the quantification of the concentration of test article in dosing solutions and in plasma samples.

Protocol	Assay Description
GTCHEM	**Transfer or Full Validation:** Many designs available to evaluate one to multiple vehicles and test articles at one time or later dates. LC/MS/MS, HPLC, GC, and UV/Vis techniques are available.

Dosing Solution Analysis

Dosing solution analysis will be conducted on the day that the samples are prepared, unless stability information allows samples to be analyzed on a different day. Upon preparation for use in each preliminary assay, each definitive assay and each confirmatory assay (as required by protocol design), samples will be collected from the vehicle, least and most concentrated dose formulations.

Protocol	Assay Description
DSA	**Dose and Vehicle Analysis:** Protocols available for every dose in all GLP assay types. LC/MS/MS, HPLC, GC, and UV/Vis techniques are available.

Stability

BioReliance will provide stability analysis of plasma or a test article under desired conditions prior to sample submission.

Protocol	Assay Description
STABIL	**Dose and Storage Condition Analysis of Test Articles:** BioReliance will reanalyze the highest and/or lowest from one set of dosing formulations after at least 3 hours storage at room temperature (15 to 30°C). The stability analysis will be conducted identically to the dosing formulation analysis for that assay, using HPLC or GC.
TKSTABIL	**Plasma or Metabolite Analysis:** BioReliance will determine stability of plasma or metabolites in plasma under the following conditions: bench top, freeze/thaw, long-term frozen, using LC/MS/MS.

Toxicokinetics/Pharmacokinetics (TK/PK)

BioReliance offers a fully validated, state-of-the-art GLP analytical service using Liquid Chromatography–Mass Spectrometry (LC/MS/MS). This powerful technology provides quantitative analysis of chemicals within a given sample, with high sensitivity and selectivity. In addition to its application as a more sensitive analytical process, LC/MS is employed for toxicokinetic (TK/PK) studies.

Protocol	Assay Description
BIOA	**Bioanalysis:** Analysis of plasma samples from in vivo studies on LC/MS/MS
TKA	**TK Analysis Report:** Fully audited report on Bioanalysis , including PK/TK calculations with tables
PLSM	**Plasma Sample Collection:** dose satellite group of animals (3/sex/group/timepoint), collect, process, and ship samples for TK analysis.

REACH Assays

Although many designs are OECD compliant and therefore are applicable for analysis of chemicals for REACH, BioReliance has selected the most pertinent assays and designs and instituted special REACH protocols. BioReliance experts are available to assist in the selection of the best options. REACH assays have specialized/streamlined protocols and reports. For questions, please contact a BioReliance representative.

Bacterial Mutation Assays (Ames)

Protocol*	Assay Description
502REACH	**ICH/OECD protocol - Plate incorporation method:** Preliminary toxicity assay and one mutation assay.
503REACH	**OECD protocol - Plate incorporation method with two trials:** One toxicity-mutation assay and a confirmatory mutation assay.
504REACH	**OECD protocol – Preincubation method:** Preliminary toxicity assay and one mutation assay.

In Vitro Gene Mutation Assay in Mammalian Cells

Protocol	Assay	Assay Description
704REACH	**Mouse Lymphoma**	**ICH/OECD protocol - Duplicate cultures per dose level:** Treatments of 4 and 24 hours without S9 and 4 hours with S9.
782REACH	**CHO/HPRT**	**OECD protocol - Duplicate cultures per dose level.** Treatment of 5 hours with and without S9.

Morphological Transformation

Protocol	Assay Description
308REACH	**In Vitro Transformation of Syrian Hamster Embryo Cells – 7 Day Exposure**

In Vivo DNA Damage and Repair

In Vivo/In Vitro Unscheduled DNA Synthesis (UDS)

Protocol	Assay Description
381REACH	**Rat Liver UDS Assay:** 40 animals dosed, 30 animals evaluated. 3 dose levels, 1 dose administration, 2 harvests.

In Vitro Cytogenetics

Chromosomal Aberrations in Human Peripheral Blood Lymphocytes

Protocol	Assay Description
341REACH	**ICH/OECD protocol (HPBL) -** Treatments of 4 and 20 hours without S9 and 4 hours with S9; cells collected at 20 hours. Numerical and structural aberrations analyzed in a minimum of 3 dose levels in duplicate. Includes mitotic inhibition as a measure of toxicity

Chromosomal Aberrations in Chinese Hamster Cells

Protocol	Assay Description
331REACH	**ICH/OECD protocol (CHO or CHL) -** Treatments of 4 and 20 hours without S9 and 4 hours with S9; cells collected at 20 hours. Numerical and structural aberrations analyzed in a minimum of 3 dose levels in duplicate. Includes cell growth inhibition, utilizing relative increase in cell counts (RICC) as a primary measure of toxicity and mitotic index as a secondary measure of toxicity.

In Vitro Micronucleus Assay

Protocol	Assay Description
348REACH	**In Vitro Micronucleus Assay in HPBL:** Treatments of 4 and 24 hours without S9 and 4 hours with S9, 9 doses for toxicity assessment, minimum of three dose levels evaluated for MN induction, 2000 cells scored per dose level. Scoring by microscopy.
368REACH	**In Vitro Micronucleus Assay in CHO Cells:** In situ method, treatments of 4 and 24 hours without S9 and 4 hours with S9, 9 doses for toxicity assessment, minimum of three dose levels evaluated for MN induction, and 2000 cells scored per dose level. Scoring by microscopy, with option for flow cytometric evaluation (please inquire).

In Vivo Cytogenetics

Chromosome Aberration Analysis in Rodents

Protocol	Assay Description
108REACH	**ICH/OECD protocol - Mouse bone marrow:** Single administration, two collection time points, vehicle controls at each time point, 100 metaphase cells evaluated/animal, 35 mice/sex. Numerical aberrations evaluated at all dose levels. Protocol 108M: males only
107GLPREACH	**ICH/OECD protocol - Rat bone marrow:** Single administration, two collection time points, vehicle controls at each time point, 100 metaphase cells evaluated/animal, 35 rats/sex. Numerical aberrations evaluated at all dose levels. Protocol 107M: males only

In Vivo Micronucleus Assay

Protocol #	Assay Description
Mice	
123012REACH	**Mouse bone marrow - ICH/OECD protocol (Microscopic Scoring):** Single administration, two collection time points, vehicle controls at each collection time point, 2000 PCE evaluated per animal, 35 mice/sex. Protocol 123M012: males only
Rats	
125012REACH	**Rat bone marrow - ICH/OECD protocol (Microscopic Scoring):** Single administration, two collection times, vehicle controls at each collection time point, 2000 PCE evaluated per animal, 35 rats/sex. Protocol 125M012: males only

Information and Ordering

For additional information or to place studies, please contact:

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