BioReliance

Toxicology Services

# 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.

## What is Big Blue®?

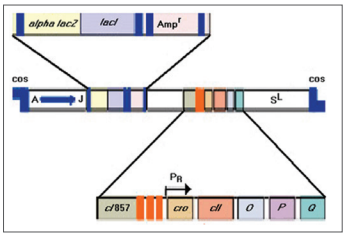
Big Blue® mice are bred to have multiple copies of recoverable target genes integrated into their genome. Big Blue® animals are created by a microinjection of the lambda shuttle vector containing the *cII* gene into the pronucleus of fertilized eggs from either C57Bl/6 mice or Fisher 344 rats.

## Why the Big Blue® Transgenic Rodent Mutation Assay?

The Big Blue® TRM assay provides a means to measure mutations in any somatic cell or germ cells. Most other in vivo assays measure DNA damage, but DNA damage does not always result in mutations. The Big Blue® TRM assay thus provides the advantage of specifically measuring gene mutations in vivo and is valuable as a second tier assay to investigate positive results from in vitro gene tox assays. TRM has been included in various regulatory guidelines and expert recommendations..

## What is the Big Blue® Transgenic Rodent Mutation Assay?

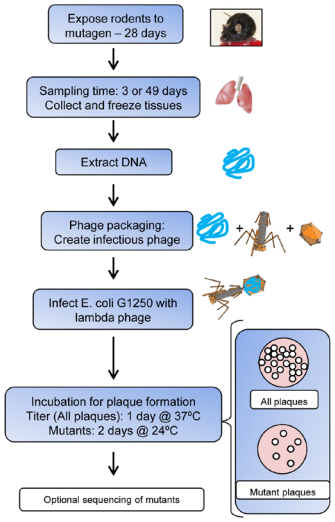
The Big Blue® Transgenic Rodent Mutation Assay (TRM) is an in vivo mutation assay currently available in mice. Gene mutation assays are recommended for regulatory submission, as they specifically measure gene mutations, not just DNA damage as other frequently used assays. BioReliance was the original validation laboratory for this assay in conjunction with the Institute of Environmental Health Sciences (NIEHS) and today has qualified the design in accordance with OECD Test Guideline 488.




The principle of the assay is that you can recover the lambda shuttle vector from genomic DNA and measure mutations in the lambda *cII* gene..

## Big Blue® Assay Overview

In a typical study, animals are dosed by oral gavage for 28 days. Tissues are then collected at day 31 after a three day period allowing DNA lesions to be fixed into the DNA and expressed as stable mutations. After collection, high molecular weight DNA is extracted from tissues of interest and purified, the shuttle vector recovered, packaged into phage, infected onto *E. coli*, and plated for plaque formation at two temperatures. *cII* mutant phage form plaques only at 24°C while all phage form plaques at 37°C. Mutation frequency is denoted by dividing the number of mutant plaques over total number of plaques. Bioavailability (TK) measurements can be added to demonstrate exposure. OECD Test Guideline 488 allows for differing designs, so please consult a BioReliance representative to design a study that will best fit your needs and the regulatory guidelines.




### Fully Qualified, Commercial Assay

Co-developed and validated by BioReliance with NIEHS in the 1990s. The assay has been re-qualified according to applicable regulatory guidelines

Approved Guideline and Regulatory Acceptance

- OECD Test Guideline 488 has been approved
- Recommended by ECHA for REACH registration
- Indicated by ICH S2(R1) and other guidelines as a follow-up to positive in vitro mutation assays

### Why BioReliance and Big Blue®?

- Over 20 years experience with TRM assay by same Study Director
- Comprehensive understanding and assays for follow-on to positive test systems
- Rapid study initiation from time of inquiry
- High efficiency of mutation detection can potentially reduce the number of assays required

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## Big Blue® Transgenic Rodent Mutation Assay

### Re-qualification Data

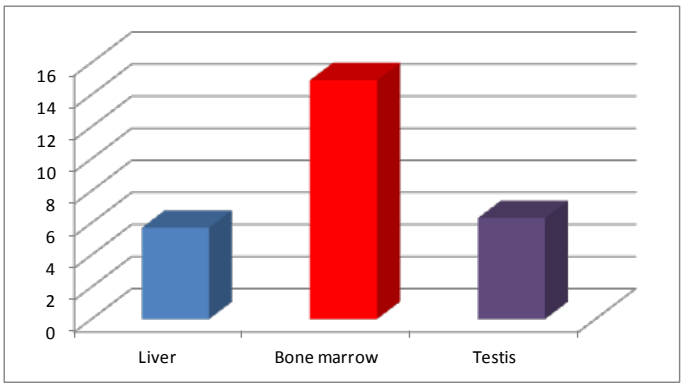
### Mutant Frequency Data

#### Representative Animal and Group (Liver)

| | Animal # | Phage Screened | # Mutants | Mutant Frequency (X $10^{-06}$) | # Packagings | Group Mean and SD (X $10^{-06}$) |
|---|---|---|---|---|---|---|
| Vehicle Control | 4101 | 978400 | 34 | 34.8 | 2 | |
| | 4102 | 1750000 | 46 | 26.3 | 2 | |
| | 4103 | 916700 | 27 | 29.5 | 2 | |
| | 4104 | 1036700 | 36 | 34.7 | 2 | |
| | 4105 | 1351700 | 58 | 42.9 | 2 | |
| | 4106 | 1835100 | 66 | 36.0 | 3 | |
| | 4107 | 2311700 | 78 | 33.7 | 2 | |
| | 4108 | 1816700 | 49 | 27.0 | 3 | 33.1 ± 5.4* |
| ENU Treatment | 4117 | 1468400 | 156 | 106 | 2 | |
| | 4118 | 1300000 | 191 | 147 | 2 | |
| | 4119 | 628400 | 167 | 266 | 2 | |
| | 4120 | 1185000 | 216 | 182 | 2 | |
| | 4121 | 1608300 | 214 | 133 | 2 | 182.0 ± 59.6* |
| ENU Treatment | 4127 | 658400 | 136 | 207 | 2 | |
| | 4128 | 563400 | 156 | 277 | 2 | |
| | 4129 | 1136700 | 208 | 183 | 2 | |
| | 4130 | 1396700 | 242 | 173 | 2 | |
| | 4131 | 1076600 | 227 | 211 | 2 | 210.1 ± 40.5* |
| ENU Treatment | 4137 | 1398300 | 270 | 193 | 2 | |
| | 4138 | 1465000 | 254 | 173 | 2 | |
| | 4139 | 830000 | 167 | 201 | 3 | |
| | 4140 | 648400 | 163 | 251 | 3 | |
| | 4141 | 1593300 | 197 | 124 | 2 | 188.5 ± 46.3* |

*Mutant frequency was homogeneous within and between groups and ENU induced statistically significant increase in *cII* mutants relative to vehicle control ($p<0.0001$)

#### Fold Increase

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