BioReliance

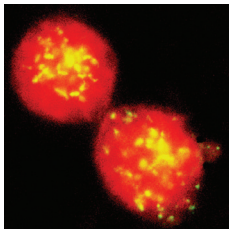
Toxicology Services

Micronucleus Assay: Cytogenetic Analysis Options

Assessment of Aneugenic or Clastogenic Mode of Action

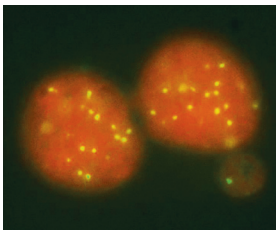
BioReliance offers FISH and CREST labeling to investigate the Mode of Action for a test article that is positive in a micronucleus assay. FISH and CREST labeling identify whether a micronuclei contains a whole chromosome(s), as a result of an aneugenic mechanism, or a chromosome fragment(s) as a result 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 and localize the presence or absence of specific centromeric DNA sequences on chromosomes. FISH uses pan-centromere probes with fluorescent labeling that bind to only those parts of the chromosome with which they show a high degree of sequence complementarity.

CREST Labeling



CREST stands for Calcinosis, Raynaud's phenomenon, Esophageal dysfunction, Sclerodactyly, and Telangiectasias. CREST uses a human autoimmune antibody that binds to kinetochores of chromosomes across species.

FISH or CREST Method - Choose The Most Suitable

Both techniques offer a qualitative study that provide results allowing a decision to be made concerning possible harmful effects of a candidate compound. These techniques evaluate the specific positive doses to look for the origin of micronuclei (clastogenic or aneugenic). If a compound proves to be aneugenic, this may be due to damage to the cellular machinary involved in segregating chromosomes during cell division, not to damage to DNA. As a result, a threshold may be able to be identified leading to the setting of safe doses for the test compound.

When applicable, FISH is the preferred method. FISH offers a more sensitive and accurate detection of centromeres. However, FISH can only be used when there are proper probes available. At this time, there are only probes for human and mouse cells.

Choose FISH when:

- Human (e.g. in vitro/HPBL) or Mouse (e.g. bone marrow)
- Can not perform on CHO cells or with Rats

Choose CREST when:

- CHO (in vitro) or Rat (in vivo)

CREST available for all options and test systems

BioReliance offers the most comprehensive commercial offerings for the investigation of positive genetox results

Some advantages of using FISH or CREST analysis:

- Provides mode of action information to help de-risk a positive micronucleus result, thus enabling regulatory approval
- Use of test method for mode of action included in OECD guidelines, and accepted by regulatory agencies
- Save valuable product development time
- Prevent wasteful use of test articles/chemicals

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FISH and CREST Ordering Information

Numerous options exist to investigate the mode of action of test articles in conjunction with the Micronucleus Assay. Options are listed below depending on what test system is preferred and whether or not a positive result and specific dose levels are known. Please consult a BioReliance representative for guidance.

FISH Assay Options

CRM Designation	Assay Name	Test System	Standard Assay Design
349FISH.BTL	In Vitro Micronucleus Assay in Human Peripheral Blood Lymphocytes with FISH option preparation	HPBL	Two 5 mL cultures, treatments of 4 hours +S9 and 24 hours -S9, 15 doses, score 3, 500 cells scored, in duplicate. Slides prepared for FISH option
349FISHselect.BTL	In Vitro Micronucleus Assay in Human Peripheral Blood Lymphocytes with FISH option preparation in select doses	HPBL	Two 5 mL cultures, treatments and doses as selected from previous MN assay with positive results, score all selected doses, 500 cells scored, in duplicate. Slides prepared for FISH option
348FISH.BTL	In Vitro Micronucleus Assay in Human Peripheral Blood Lymphocytes with FISH option preparation	Human Peripheral Blood Lymphocytes (HPBL)	Preliminary toxicity assay, treatments of 4 hours +S9 and 4 and 24 hours -S9, 24 hour collection, 9 doses, score minimum of 3, 2000 cells scored per dose level (triplicate cultures per treatment). Slides prepared for FISH option
348FISHselect.BTL	In Vitro Micronucleus Assay in Human Peripheral Blood Lymphocytes with FISH option preparation in select doses	Human Peripheral Blood Lymphocytes (HPBL)	Limited treatments and doses as chosen from previous MN assay with positive results. Score all selected doses, 2000 cells scored per dose level (triplicate cultures per treatment). Slides prepared for FISH option
Additional Preparation for In Vivo FISH	Preparation for In Vivo FISH Option (added to In Vivo Mouse MN Assay)	Mouse Bone Marrow	Aneugenic positive control added. Filter bone marrow cells through cellular column Prepare and store slides at -30°C for potential FISH
FISHselect.BTL	In Vivo Micronucleus Assay with FISH option preparation in select doses	Mouse Bone Marrow	Definitive assay with select doses. Extra slides prepared for FISH option and stored at -30°C
FISH_001.BTL	In Vitro Micronucleus Option - Fluorescent In Situ Hybridization (FISH)	Slides	Slides labeled (stained) for up to 3 treatment conditions. Evaluation of dose level statistically positive for the presence of micronuclei
FISH_002.BTL	In Vivo Micronucleus Option - Fluorescent In Situ Hybridization (FISH)	Slides	Process slides from cultures selected for FISH analysis. Scoring for centromere positive micronuclei. Evaluate and report FISH data (Mouse Only)

CREST In Vitro Options

CRM Designation	Assay Name	Test System	Standard Assay Design
349CREST.BTL	In Vitro Micronucleus Assay in Isolated Human Peripheral Blood Lymphocytes with CREST option preparation	HPBL	Two 5 mL cultures, treatments of 4 hours +S9 and 24 hours -S9, 15 doses, score 3, 500 cells scored, in duplicate. Slides prepared for CREST option
349CRESTselect.BTL	In Vitro Micronucleus Assay in Isolated Human Peripheral Blood Lymphocytes with CREST option preparation in select doses	HPBL	Two 5 mL cultures, treatments and doses as selected from previous MN assay with positive results, score all selected doses, 500 cells scored, in duplicate. Slides prepared for CREST option
369CREST.BTL	In Vitro Micronucleus Assay in CHO Cells with CREST option preparation	CHO cells	3 cultures, treatments of 4 hours +S9 and 24 hours -S9, 15 doses, score 3, 1000 cells scored per dose. Slides prepared for CREST option
369CRESTselect.BTL	In Vitro Micronucleus Assay in CHO Cells with CREST option preparation in select doses	CHO cells	3 cultures, treatments and doses as selected from previous MN assay with positive results, score all selected doses, 1000 cells scored per dose. Slides prepared for CREST option
348CREST.BTL	In Vitro Micronucleus Assay in Isolated Human Peripheral Blood Lymphocytes with CREST option preparation	Human Peripheral Blood Lymphocytes (HPBL)	Preliminary toxicity assay, treatments of 4 hours +S9 and 4 and 24 hours -S9, 24 hour collection, 9 doses, score minimum of 3, 2000 cells scored per dose level. Slides prepared for CREST option
348CRESTselect.BTL	In Vitro Micronucleus Assay in Isolated Human Peripheral Blood Lymphocytes with CREST option preparation in select doses	Human Peripheral Blood Lymphocytes (HPBL)	Limited treatments and doses as chosen from previous MN assay with positive results. Score all selected doses, 2000 cells scored per dose level. Slides prepared for CREST option
368CREST.BTL	In Vitro Micronucleus Assay in CHO Cells with CREST option preparation	Chines Hamster Ovary Cells (CHO)	Preliminary toxicity assay, treatments of 4 hours +S9 and 4 and 24 hours -S9, 24 hour collection, 3 cultures, score minimum of 3 dose levels for micronucleus induction, 2000 cells scored per dose level. Slides prepared for CREST option
368CRESTselect.BTL	In Vitro Micronucleus Assay in CHO Cells with CREST option preparation in select doses	Chines Hamster Ovary Cells (CHO)	Limited treatments and doses as chosen from previous MN assay with positive results. Score all selected doses, 2000 cells scored per dose level. Slides prepared for CREST option
CREST_004.BTL	In Vitro Micronucleus Option - Anti-Kinetochore Labeling	Slides	Slides stained for up to 3 treatment conditions and PCs. Evaluation of dose level(s) statistically positive for the presence of micronuclei
CREST_001.BTL	In Vitro Micronucleus Option - Anti-Kinetochore Labeling	Slides	Slides stained for up to 3 treatment conditions and PCs. Evaluation of dose level(s) statistically positive for the presence of micronuclei

CREST In Vivo Options

CRM Designation	Assay Name	Test System	Standard Assay Design
Additional Preparation for In Vivo CREST	Preparation for In Vivo CREST Option (added to In Vivo MN Assay)	Rodent Bone Marrow	Aneugenic positive control added. Filter bone marrow cells through cellular column Prepare and store slides at -30°C for potential CREST
CRESTselect.BTL	In Vivo Micronucleus Assay with CREST option preparation in select doses	Rodent Bone Marrow	Definitive assay with select doses. Extra slides prepared for CREST option and stored at -30°C
CREST_002.BTL	In Vivo Micronucleus Option -Anti-Kinetochore Labeling	Slides	Slides stained. Evaluation of dose level statistically positive for the presence of micronuclei

In Vitro Non-GLP Assays
In Vitro GLP Assays
In Vivo GLP Assay
In Vitro FISH Option
In Vivo FISH Option

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