Micronuclei MGP_MIC_001

Purpose

The micronuclei assay is a mutagenic test that can identify increases in the frequency of normochromatic micronucleated erythrocytes, reticulocytes and micronucleated reticulocytes, all of which can act as markers for chromosomal damage. The assay can detect genotoxic events that occur during interphase, which may be the result of a disruption to the number of chromosomes (aneugenic), or by damage to the chromosomes themselves (clastogenic).

Experimental Design

Minimum number of mutant animals: 7 mice for each sex.

Age of animals: 16 weeks (fixed).

Sexual dimorphism: yes for some of the parameters.

Equipment

1. 100 µl EDTA coated blood collection tubes without capillary (Scientific Laboratory Supplies catalogue number 078042)
2. Centrifuge (for plates)
3. Dispensing troughs
4. Sterilin 96 V bottom plates with lid
5. Sterilin 96 flat bottom plates with lid
6. FACS buffer (PBS with 0.5% BSA)
7. Pipettes (single, multi and repeat) and filter tips
8. DNA dye waste container, plus funnel
9. Timer
10. 4 ml amber glass vials (Sigma catalogue number 27001-U)
11. BD LSRII flow cytometer
12. APC-Terr 119, PE-CD71 antibodies and Hoechst dye

Procedure

Sample collection and preparation:

a. 49 µl of FACS buffer is pipetted into the required wells of a 96 well V bottom plate

b. 1 µl of whole blood from a 100 µl EDTA coated tube (collected by puncture of the retro-orbital sinus) is pipetted onto the plate. This is repeated for all samples.

c. 50 µl of 2x CD71/Ter119/Hoechst cocktail is added and incubated for 20 minutes.

d. Cells are washed with FACS buffer before 25µl of the stained sample is transferred to a flat bottom plate containing 275 µl of FACS buffer. The plate can then be run on the BD LSRII flow cytometer.

Analysis

Data is analysed using FlowJo software. PE-A CD71 is plotted against Hoechst-A to distinguish and gate reticulocyte (RET), micronucleated reticulocyte (MN-RET), normochromic erythrocyte (NCE) and micronucleated normochromic erythrocyte (MN-NCE) populations.

Notes

The micronucleus assay is performed at 16 weeks of age

Non-fasted mice are terminally anaesthetised and blood is collected into EDTA coated tubes via the retro-orbital sinus. Whole blood is stained with a titrated cocktail of antibodies.

In order to eliminate aggregated antibodies from the mix, briefly centrifuge the antibodies before adding them to the cocktail.

Parameters and Metadata

% MN-NCE MGP_MIC_001_001 | v1.0

simpleParameter

Req. Analysis: false **Req. Upload:** true **Is Annotated:** true

Unit Measured: %

% MN-RET MGP_MIC_002_001 | v1.0

simpleParameter

Req. Analysis: false **Req. Upload:** true **Is Annotated:** false

Unit Measured: %

% RET MGP_MIC_003_001 | v1.0

simpleParameter

Req. Analysis: false **Req. Upload:** true **Is Annotated:** true

Unit Measured: %

FACS equipment manufacturer MGP_MIC_004_001 | v1.0

procedureMetadata

Req. Analysis: true **Req. Upload:** true **Is Annotated:** false

Options: BD Biosciences, Beckton Dickinson, Beckman Coulter,

FACS equipment name MGP_MIC_005_001 | v1.0

procedureMetadata

Req. Analysis: true **Req. Upload:** true **Is Annotated:** false

Options: Flow cytometer,

FACS equipment model MGP_MIC_006_001 | v1.0

procedureMetadata

Req. Analysis: true **Req. Upload:** true **Is Annotated:** false

Options: LSR II, FC500, LSR Fortessa X20,

FACS sample status MGP_MIC_007_001 | v1.0

procedureMetadata

Req. Analysis: true **Req. Upload:** true **Is Annotated:** false

Options: Fresh, Fixed, Analysed next day unfixed, Prepared next day, analysed fresh,

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