TEST CODE:
CT-089

Eye Irritation Test

OCULAR IRRITECTION® (*In vitro* Macromolecular Test Method for Identifying Chemicals Inducing Serious Eye Damage and Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage) (OECD TEST GUIDELINE 496)

OVERVIEW

Corneal opacity is an important driver for the classification of eye hazards. Changes to corneal opacity (a precursor of irritation and serious eye damage) can be caused by disruption of the highly organised structure of the corneal proteins and carbohydrates.

The test system used for this method contains a macromolecular reagent composed of a mixture of proteins, glycoproteins, carbohydrates, lipids, and low molecular weight components. When rehydrated, this mixture forms a complex macromolecular matrix which mimics the highly ordered structure of the transparent cornea. Test items that are hazardous will produce turbidity of the macromolecular reagent through promoting protein denaturation, unfolding, and changes in conformation (as well as disruption and disaggregation of the macromolecular matrix components).

Before proceeding to the main test, the item to be tested is characterised through determining the pH of a 10% dilution in water and assessing whether the product is a surfactant. Samples with a pH ≥4 or ≤9 are suitable to be tested by this method. Certain items are not suitable to be tested by this method. These include intensely coloured chemicals, substances which cause salting out precipitation, high concentrations of surfactants, and highly volatile substances.

During the main test, based on their physico-chemical properties, items are applied directly to the macromolecular matrix at room temperature, or onto a membrane disc above the matrix. Items without surfactant-like properties are tested with a series of five doses (125, 100, 75, 50, 25 µl (liquids)/mg (solids)) applied neat to the membrane disc. Items with surfactant-like properties are diluted in sterile water and 125 µl applied directly to the macromolecular matrix at concentrations of 5%, 2.5%, 1.25%, 0.625%, 0.3125%, then covered with the membrane. Waxy solids are applied directly to the matrix then covered with the membrane disc.

The matrix is exposed for 24hr ± 0.5hr at 25°C±1°C, and visually inspected for damage to the membrane disc. The contents are transferred to a 96 well plate,

TURNAROUND TIME	*Results in 4 weeks. (6-8 weeks including GLP report)*
AMOUNT OF SAMPLE REQUIRED	*10ml (liquids) / 10g (solids). Please enquire if sample availability is limited.*
PRICE	*Please enquire. Reduced pricing may be available for multiple items.*
FURTHER DOWNLOADS	XCellR8 Good Laboratory Practice (GLP) Compliance Certificate.

and their optical density (OD) recorded at 405nm using a spectrophotometer.

The OD readings recorded are used to calculate an Irritection® Draize Equivalent Score (IDE) for each item tested. A Maximal Qualified Score (MQS) is determined and represents the highest IDE score obtained from various tested concentrations of an item. The MQS can range from 0 to 51, and is used to predict the UN GHS classification. According to the OECD Test Guideline, the method is validated to predict No Category, or Category 1. Where the MQS would indicate a classification between these, a result of No Prediction is obtained, and further testing will be required to determine a category.

TEST SYSTEM

The test system consists of two components: a macromolecular matrix and a membrane disc for the controlled delivery of the test chemical to the macromolecular matrix. The macromolecular reagent is composed of a mixture of proteins, glycoproteins, carbohydrates, lipids, and low molecular weight components. When rehydrated, this mixture forms a complex macromolecular matrix which mimics the highly ordered structure of the transparent cornea. The macromolecular gel matrix serves as the target for the test substance - protein oligomers which are part of the matrix self-associate to form larger fibrils that are held together by non-covalent forces. Chemicals that cause ocular damage denature the proteins causing disaggregation of the matrix and turbidity of the macromolecular reagent.

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SUMMARY OF THE TEST METHOD

- Prior to the main study, the test item undergoes preliminary checks for pH and potential as a surfactant.
- Items are applied directly to the macromolecular matrix at room temperature, or over a cellulose membrane.
- Items without surfactant-like properties are tested with a series of five doses (125, 100, 75, 50, 25 µl (liquids) /mg (Solids)) applied neat to the membrane disc.
- Items with surfactant-like properties are diluted in sterile water and 125 µl applied to the disc at concentrations of 5%, 2.5%, 1.25%, 0.625%, 0.3125%.
- Waxy solids are applied directly to the reagent solution then covered with the membrane disc.
- The matrix is exposed for 24hr ± 0.5hr at 25°C±1°C and visually inspected.
- The contents of plates are transferred to a 96 well plate, and their optical density (OD) recorded at 405nm using a spectrophotometer.
- The OD readings recorded are used to calculate a Irritection® Draize Equivalent Score (IDE) for each item tested. A Maximal Qualified Score (MQS) is determined and represents the highest IDE score obtained from various tested concentrations of an item.
- The MQS is used to predict the UN GHS classification.

WHY CONDUCT AN OCULAR IRRITECTION TEST?

Ocular Irritection® is a biochemical *in vitro* test that investigates the potential of a substance to cause serious damage, or no irritation to the eye. Eye irritation is a reversible condition caused by changes to the surface of the eye, whereas serious eye damage is not expected to be reversible within 21 days. This method models changes to corneal opacity by observing the effect of a test chemical on a macromolecular reagent matrix. The test can determine whether a substance causes serious eye damage or has no required classification for irritation or eye damage, and is an effective first step for a top-down or bottom-up approach to testing as defined in the OECD's Guidance Document 263: Integrated Approaches to Testing and Assessment (IATA) for Serious Eye Damage and Eye Irritation.

QUALITY STATEMENT

XCellR8 is accredited by the UK Medicines and Healthcare Products Regulatory Authority (MHRA) for the conduct of *in vitro* safety testing in compliance with Good Laboratory Practice (GLP). This means that we are able to provide you with test results that may be used at a regulatory level to demonstrate product safety, where the test is an approved regulatory method. The test method described here is non-regulatory but is conducted in our GLP-accredited laboratory.

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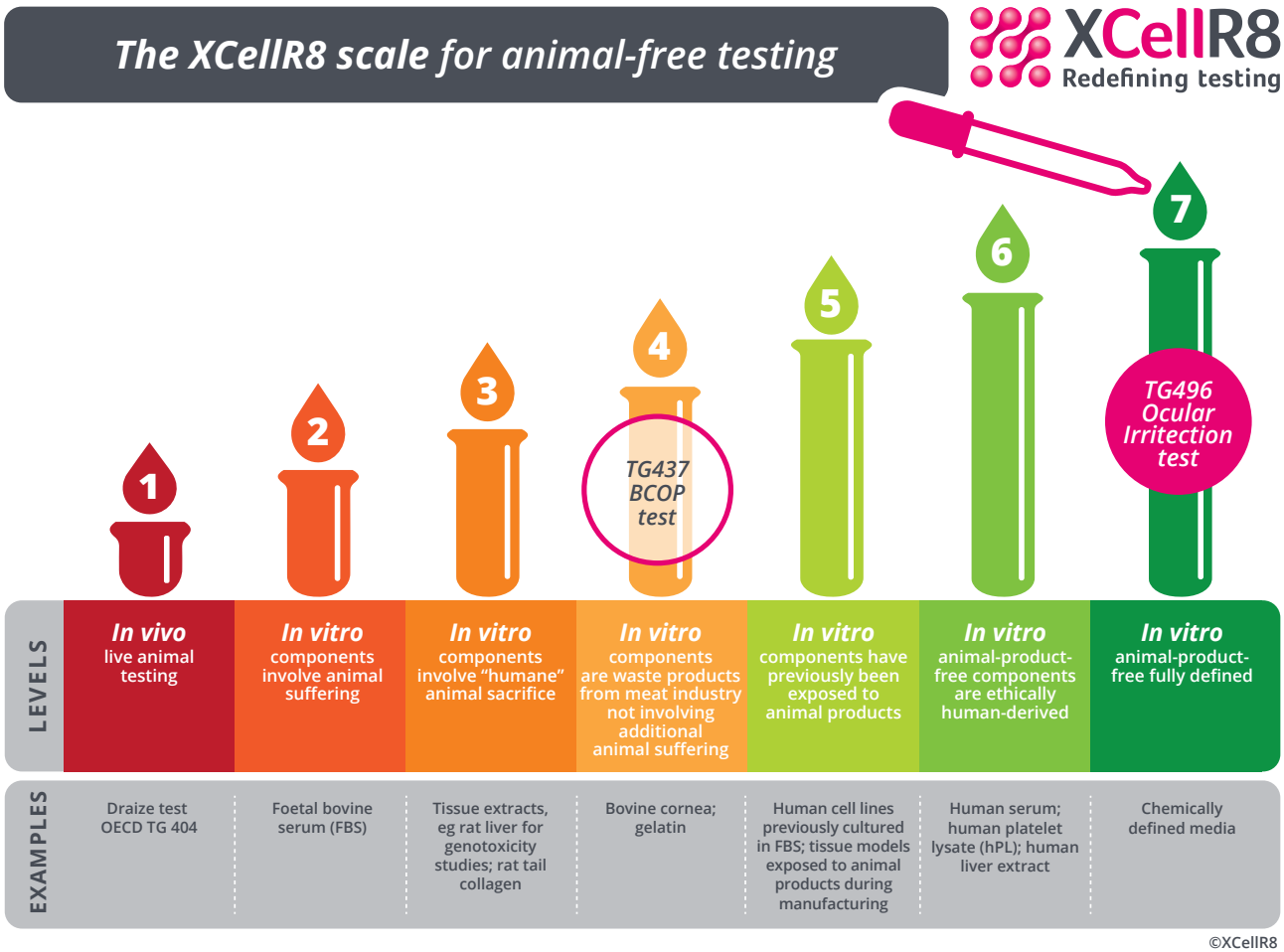
ETHICAL ASSESSMENT

The Ocular Irritection® test has undergone ethical assessment at XCellR8 according to our published 7-point scale for animal-free testing. The test is a fully defined system, free of any animal or human derived components, and therefore achieves the highest ethical accreditation – Level 7 on the scale.

The Ocular Irritection® test is an alternative to the Bovine Corneal Opacity and Permeability (BCOP) test (OECD Test Guideline 437), providing an equivalent level of scientific data and regulatory acceptance, but without the need to use bovine corneas from slaughterhouses. (For comparison, the BCOP test is classified only as Level 4 on our 7-point scale).



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