

AUTOMATED GENOTOX SOLUTION

AUTOMATED DETERMINATION OF THE GENOTOXIC POTENTIAL





THE FUTURE OF GENOTOX

The FADU assay (basis for AUREA gTOXXs)

The detection of DNA strand breaks and repair is based on progressive DNA unwinding under highly controlled conditions of alkaline pH, time and temperature. DNA "open sites" are the starting points for the unwinding process. They occur naturally during cell cycle processes at replication forks or the ends of chromosomes. As yet, they may additionally be induced by reactive oxygen species, irradiation or genotoxic chemicals. The assay is based on a sensitive fluorescent probe which serves as marker for the loss of intact double stranded DNA. (Decrease in the fluorescence intensity indicates an increase in DNA unwinding and consequently a greater number of DNA strand breaks).

Advantages of AUREA gTOXXs

The AUREA gTOXXs Analyzer speeds up your risk assessment of test substances, as well as the DNA repair capacity in various human cells lines, by use of the AUREA assay (the automated FADU assay). The automation of DNA strand break detection enables reliable determination of the genotoxic potential of chemical, nanoparticle or biological substances.

The gTOXXs operation time, reproducibility and workflow routine is clearly superior to other established genotoxicity assays, like the COMET assay. At low costs, robustness is optimal due to high sensitivity and precision. DNA strand breaks are reliably detected even after short cell exposure (≥10 min) to well-known genotoxic compounds at low concentrations (Moreno-Villanueva et al, 2011). The rapid assessment of the genotoxic potency will significantly enhance product safety testing in scientific as well as commercial large-scale projects. With the validation of the gTOXXs for 3D skin models completely new applications become available.

| | ® ₀ 0 0 | | △ ¾ | 1 | △ ¾ | 1 | (n) 9 | Control values: |
|------------------------------|--------------------|---------|-----|--------|-----|-------------------|--|---|
| No DNA-damage (Control 1) | | lysis | 33 | neutr. | 33 | NaOH SybrGreen | \$ \$ | Absolute DNA quanti |
| No DNA-damage (Control 2) | | lysis | | NaOH | SE, | neutr. | X | Control values: Physiological unwind endogenous DNA dar |
| DNA-damage (middle dose) | | lysis > | 38 | NaOH | ZZ | neutr. | XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX | Values for various grades of damage |
| DNA-damage (high dose) | | lysis | 是是 | NaOH | きたい | neutr. | をいた | SybrGreen® background |

| COMET assay | | AUREA assay | | | | |
|---|------------|-------------|-------------|---|----|--|
| STEPS | TIME (min) | | TIME (min) | STEPS | RC | |
| Buffer preparation: aliquots possible | 5 | | 5 | Buffer preparation: aliquots | | |
| Agarose preparation: Distribution in Eppendorf tubes | 20 | | | Equipment: Switch on the robot | | |
| Equipment: assemble gel electrophoresis device | 15 | 15 50 | 2 | | | |
| Eppendorf tubes with agarose at 37 °C | 15 | | • | | | |
| DNA damage infliction | 30 | | 30 | DNA damage infliction | | |
| Embedding cells in agarose on the slides | 60 | | | Automatic steps: Additon of suspension buffer transfer of samples to the 96-well plate, lysis buffer, alkaline buffer, neutralization buffer, Fluorescent Dye | | |
| Cool down agarose | 10 | >510 | | | | |
| Lysis buffer | 30 | | : | | | |
| Alkaline buffer | 60 | | 120 | | | |
| Electrophoresis | 30 | | . 120 | | | |
| 70% Ethanol | 5 | | : | | | |
| Drying of the slides | 60 | | : | | | |
| SybGreen | 15 | | : | | | |
| SybGreen incubation | >240 | | : | | | |
| Microscopy | >60 | | 2 | Fluorescence reader | | |
| Data analysis | >60 | | 10 | Data analysis | | |
| TOTAL TIME | >7: | 15 | ONLY 174 | TOTAL TIME | | |

Applications of the AUREA gTOXXs Analyzer

- DNA damage detection-Dose-response relationship
- DNA repair after defined induction of damage
- DNA interstrand crosslinks
- Oxidative DNA lesions
- Post-traumatic stress disorder (PTSD)
- Individualized cancer therapy (chemotherapy and radiation therapy)
- Ageing

gTOXXs provides a true alternative to animal testing.







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