KAMIYA BIOMEDICAL COMPANY

Oxidative DNA Damage Kit, Qualitative

Fluorescent Assay for the Qualitative Detection of 8-oxoguanine in DNA

Cat. No. DN-001

For Research Use Only. Not for Use in Diagnostic Procedures.

PRODUCT INFORMATION

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INTENDED USE

The **K-*ASSAY*®** Oxidative DNA Damage Kit, Qualitative, is an *in vitro* fluorescent protein binding method for the detection of oxidative damage to DNA in fixed permeabilized cells using the Fluorescent Activated Cell Sorting (FACS) technique. Material of both human and animal origin can be tested.

Oxidative DNA damage can be found in most cell types and can be caused by many different mechanisms. Because of this, the instructions given in this booklet should only be regarded as guidelines and the procedures may need to be modified according to the experimental model or cell type being studied. The **K-*ASSAY*®** Oxidative DNA Damage Kit, Qualitative is intended for *in vitro* research use only. Not for use in diagnostic procedures.

BACKGROUND

Oxidative injury to macromolecules is indicated in a wide range of pathological conditions. Damage is mediated via free radicals that can be created by a range of agents such as xenobiotics, environmental toxins, radiation, smoking, ischemia-reperfusion injury oxidising agents and normal or disturbed metabolic activity. These free radicals may react with DNA causing reversible and irreversible damage. This can lead to mutation, carcinogenesis, teratogenesis or cell death.

The probe in the **K-*ASSAY*®** Oxidative DNA Damage Kit, Qualitative is specific for 8-oxoguanine. 8-oxoguanine (as part of the oxidized nucleotide 8-oxyguanosine) is formed during free radical damage to DNA and is a sensitive and specific indicator of oxidative DNA damage. 8-oxoguanine is a particularly important biomarker of oxidative DNA damage as it is formed in relatively large quantities and 8-oxoguanine formation can lead to mutations, such as the substitution of thymine for guanine and cytosine for adenine. Previously, 8-oxoguanine was difficult to detect, requiring HPLC analysis. However, by utilizing a binding protein with high avidity and specificity for 8-oxoguanine, the **K-*ASSAY*®** Oxidative DNA Damage Kit, Qualitative is a simple, convenient, sensitive fluorescence method for detecting for oxidative DNA damage.

ASSAY PRINCIPLE

The **K-*ASSAY*®** Oxidative DNA Damage Kit, Qualitative utilizes a direct fluorescent protein binding technique. After cells have been fixed and permeabilized, the FITC labeled protein conjugate is added and binds to the 8-oxoguanine moiety present in the 8-oxoguanosine of oxidized DNA. The presence of oxidized DNA is indicated by a green/yellow fluorescence that can be read using the Fluorescence Activated Cell Sorting procedure.

KIT COMPONENTS

Each kit contains reagents sufficient for 50 determinations using standard assay procedures.

1. Wash Concentrate: 25X concentration of TRIS buffered saline/Tween-20 (TBST, 55 mL). Contains Proclin950. CONCENTRATE.
2. FITC-Conjugate: Binding Protein-FITC-Conjugate (0.5 mL). Contains ProClin950 and Bronidox L. CONCENTRATE.
3. Package Insert.

ADDITIONAL MATERIALS REQUIRED

1. Micropipettes: 20 µL to 100 µL, 200 µL to 1000 µL.
2. Test tubes.

3. 1 L beaker.
4. Graduated cylinder.
5. Reagents for fixing and permeabilizing cells.
6. Distilled/de-ionized water.
7. 37°C water bath.
8. Flow cytometer with appropriate filter combination for FITC (excitation filter 495 nm, barrier filter 515 nm).

STORAGE

1. The kit is stable until the expiration date indicated on the outer box label, provided it is stored at 4°C.
2. Prepared Wash Solution (TBST) is stable at room temperature (RT, 20-25°C) for up to two weeks. Store at 4°C if extended storage is required.
3. Avoid unnecessary exposure of FITC-Conjugate to light.

PRECAUTIONS

Safety

1. The **K-*ASSAY*®** Oxidative DNA Damage Kit, Qualitative is intended for *in vitro* research use only. Not for use in diagnostic procedures.
2. The **K-*ASSAY*®** Oxidative DNA Damage Kit, Qualitative is intended for use by qualified laboratory staff only.
3. Dispose of all specimens in accordance with good laboratory practice.
4. Wear protective clothing, disposable latex gloves and eye protection while handling specimens and performing the assay. Wash hands thoroughly when finished.
5. Do not pipette by mouth and never eat or drink at the laboratory workbench.

Procedural

1. Do not use kit or individual reagents past their expiration date.
2. Do not mix or substitute reagents from different kit lots.
3. Care must be taken not to contaminate components and always change pipette tips for each sample and component.
4. Do not use reagents that are cloudy or that have precipitated out of solution.
5. High quality distilled or de-ionized water is required for the dilution of the Wash Solution. The use of poor quality or contaminated water may lead to background color in the assay.
6. Allow all reagents to come to RT and mix well prior to use.
7. Avoid leaving reagents in direct sunlight and/or above 4°C for extended periods.
8. Always use clean, preferably disposable, glassware for all reagent preparation.

PREPARATION OF REAGENTS

Wash Solution (TBST)

Perform a 1/25 dilution of Wash Concentrate by adding, for example, 10 mL of Wash Concentrate to 240 mL de-ionized water as required. Prepare only the volume of diluted Wash Solution required for the assay.

FITC-Conjugate

Prepare a 1/10 dilution of the FITC-Conjugate Concentrate by adding, for example, 100 µL of FITC-Conjugate Concentrate to 900 µL of diluted Wash Solution.

To dispense the Conjugate, remove the dropper cap and pipette directly from the bottle. Keep the diluted FITC-Conjugate solution in the dark when not in use.

The dilution of the FITC-Conjugate has been used successfully for a number of cell lines, e.g. Hep G2, IMR-32 and CHO, sperm and lymphocytes. In some experimental situations, it may be necessary to optimize the dilution of the conjugate used. This will be dependent on the particular cell type used and the experimental conditions used to induce oxidative damage. If the incorrect concentration of the conjugate is used, high background staining and non-specific binding may occur.

SAMPLE PREPARATION AND HANDLING

Live cells should be shielded from high oxygen tensions (e.g. room air) and unnecessary mechanical stress (e.g. mixing and washing) as these can lead to increased oxidative DNA damage and high levels of fluorescence in untreated/control samples. DNA is more stable in fixed cells.

FLOW CYTOMETRY ASSAY PROCEDURE

The exact methods used will vary according to the cell type studied and flow cytometer used.

1. Fix and permeabilize cells to be tested.
2. Wash.
3. Add 100 µL FITC-Conjugate to the cell pellet and incubate in the dark for 60 minutes at RT.
4. Wash.
5. Read fluorescence in a flow cytometer using excitation wavelength of 495 nm and barrier filter of 515 nm.

PERFORMANCE CHARACTERISTICS

The ***K-ASSAY®*** Oxidative DNA Damage Kit, Qualitative is specific for the 8-oxoguanine moiety of 8-oxoguanosine present in oxidized DNA and shows no measurable cross-reactivity with unoxidized guanine/guanosine or other unoxidized nucleotides.

FOR RESEARCH USE ONLY

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