

**Catalogue No: BIO81DNA
50 determinations**



ARGUTUS MEDICAL

OxyDNA Test

**Fluorescent Protein Binding Method for
the Detection of Oxidative DNA Damage**

Instructions for use

**FOR RESEARCH USE ONLY
Not for use in Diagnostic Procedures**

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INTENDED USE

The Argutus Medical OxyDNA Test is an *in-vitro* fluorescent protein binding method for the detection of oxidative damage to DNA in fixed permeabilised 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.

SUMMARY AND EXPLANATION OF THE TEST

Oxidative injury to macromolecules is indicated in a wide range of pathological conditions¹⁻⁴. Damage is mediated via free radicals that can be created by a range of agents, e.g. xenobiotics⁵, environmental toxins⁶⁻⁸, smoking⁷, radiation⁹⁻¹³, ischaemia-reperfusion injury¹⁴ oxidising agents¹⁵ and normal or disturbed metabolic activity¹⁶. These free radicals may react with DNA causing reversible and irreversible damage. This can lead to mutation, carcinogenesis¹⁻³, teratogenesis^{5,16} or cell death.

The probe in the Argutus Medical OxyDNA Test is specific for 8-oxoguanine. 8-oxoguanine (as part of the oxidized nucleotide 8-oxoguanosine) is formed during free radical damage to DNA and is a sensitive and specific indicator of oxidative DNA damage⁹. 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 adenine³. 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 Argutus Medical OxyDNA Test is a simple, convenient, sensitive fluorescence method for detecting for oxidative DNA damage⁴⁻¹⁸.

PRINCIPLE OF THE PROCEDURE

The Argutus Medical OxyDNA Test utilizes a direct fluorescent protein binding technique. After cells have been fixed and permeabilised, 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.

COMPONENTS

Each Argutus Medical OxyDNA Test kit contains reagents sufficient for 50 determinations using standard assay procedures.

1. Wash Concentrate: 25x TRIS buffered saline/ Tween-20 (TBST 55mL).
Contains 0.01% Thiomersal. CONCENTRATE.
2. FITC-Conjugate: Binding Protein-FITC conjugate (0.5 mL).
Contains 0.01% Thiomersal. CONCENTRATE.
3. Product Insert.

PRECAUTIONS

SAFETY

- The Argutus Medical OxyDNA Test is for *in-vitro* research use only. Not for use in diagnostic procedures
- The Argutus Medical OxyDNA Test is intended for use by qualified laboratory staff only.
- The Argutus Medical OxyDNA Test contains a chemical known to the State of California to cause birth defects or other reproductive harm (California Prop 65: Thiomersal).
- Dispose of all specimens in accordance with good laboratory practice.
- Wear protective clothing, disposable latex gloves and eye protection while handling specimens and performing the assay. Wash hands thoroughly when finished.
- Do not pipette materials by mouth and never eat or drink at the laboratory workbench.

PROCEDURAL

- Do not use kit or individual reagents past their expiry date.
- Do not mix or substitute reagents from different kit lot numbers.
- Care must be taken not to contaminate components and always use fresh pipette tips for each sample and component.
- Do not use reagents that are cloudy or that have precipitated out of solution. High quality distilled or deionised water is required for the Wash Solution. The use of poor quality or contaminated water may lead to background colour in the assay.
- Allow all reagents to come to room temperature (20–25°C) and mix well prior to Use.
- Avoid leaving reagents in direct sunlight and/or above 2-8°C for extended periods.
- Always use clean, preferably disposable, glassware for all reagent preparation.

STABILITY AND STORAGE

1. All kit reagents should be stored at 2-8°C and are stable as supplied until the expiry date shown.
2. Prepared Wash Solution (TBST) is stable at room temperature for up to two weeks. Store at 2-8°C if extended storage is required
3. FITC conjugate must be stored in the dark.

ADDITIONAL MATERIALS REQUIRED

1. Micropipettes: 20µL to 100µL, 200µL to 1000µL
2. Test tubes
3. 1 L beaker
4. Graduated cylinder
5. Reagents for fixing and permeabilising cells
6. Deionised/Distilled water
7. 37°C water bath
8. Flow cytometer with appropriate filter combination for FITC (excitation filter 495 nm, barrier filter 515 nm)

See instructions of relevant flow cytometry apparatus

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. Contact Argutus Medical for advice.

PREPARATION OF REAGENTS

WASH SOLUTION (TBST)

Perform a 1/25 dilution of Wash Concentrate adding, for example, 10mL of Wash Concentrate to 240mL deionised water as required. Prepare only the volume of 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 Wash Solution.

To dispense conjugate, remove cap and pipette directly from the bottle.

Keep FITC conjugate solutions in the dark when not in use.

These dilutions of FITC conjugate have 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 optimise 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 conjugate is used, high background staining and non-specific binding may occur. Contact Argutus Medical for advice.

FLOW CYTOMETRY TEST PROCEDURE

The exact methods used will vary according to the cell type studied and Flow Cytometer used. Contact Argutus Medical for advice.

1. Fix and permeabilise 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 room temperature.
4. Wash.
5. Read fluorescence in a Flow Cytometer using excitation wavelength of 495nm and barrier filter of 515nm.

PERFORMANCE CHARACTERISTICS

The Argutus Medical OxyDNA Test is specific for the 8-oxoguanine moiety of 8-oxoguanosine present in oxidized DNA and shows no measurable cross reactivity with unoxidised guanine / guanosine or other unoxidised nucleotides.

WARRANTY

Any change or modification of the procedure, not recommended by Argutus Medical, may affect the results, in which case Argutus Medical disclaims all warranties, expressed, implied or statutory, including implied merchantability and fitness for use. In the case of such an event, Argutus Medical shall not be liable for damages, direct or consequential.

OTHER ARGUTUS MEDICAL SMARTASSAYS

Pancreatic Injury Testing Service

Catalogue No	Product Name	Description
TEST BBU	Trypsinogen Activation Peptide (TAP) EIA	TAP in human and mammalian urine and tissue

Animal Organ Damage Biomarkers

Catalogue No	Product Name	Description
BIO64RT	Rat Alpha GST EIA	α GST in rat serum, urine and tissue culture
BIO76YB1	Rat Yb1 GST EIA	GSTYb1 (μ GST) in rat urine
BIO89RPA1	RPA-1 EIA	Renal papillary antigen 1 in rat urine
BIO87CD	RPA-1 Antiserum	Antibody to rat collecting duct
BIO88LH	RPA-2 Antiserum	Antibody to rat loop of henle

Human Organ Damage Biomarkers

Catalogue No	Product Name	Description
BIO66NEPHA	NEPHKIT® Alpha GST EIA	α GST in human urine
BIO60HEPA	HEPKIT® Alpha GST EIA	α GST in human serum and plasma
BIO60HEPAS	High Sensitivity Alpha GST EIA	α GST in human serum and plasma
BIO85	PI GST EIA	π GST in human urine and plasma
BIO83	Urinary Collagen IV EIA	Collagen IV in human urine
BIO82	Serum Collagen IV EIA	Collagen IV in human serum
BIO81DNA	OxyDNA test	Fluorescence method for the detection of oxidative DNA damage in cell suspensions

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