

DNA Lesion Detection

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International Edition

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Introduction

Exposure of cells to DNA-reactive agents (exogenous and endogenous carcinogens, free radicals or cancer chemotherapeutic drugs) results in a variety of potentially mutagenic or cytotoxic modifications of genomic DNA. Antibody based methods are ultrasensitive detection techniques for the identification and quantification of these defined DNA lesions, not only in isolated DNA, but also in individual cells, or specific gene sequences. Various methods have been developed that enable to detect DNA-lesions in the femtomole to attomole range (Figure 1).

O-alkylations result from exposure to members of the large class of N-nitroso compounds (e.g. carcinogens like nitrosamines, cancer therapeutic agents, compounds of tobacco smoke). O⁶-alkylations are removed from cellular DNA by different repair systems in cell type and species specific efficiency, thus representing excellent markers for studies of formation and elimination processes. O²-alkylthymines are generated to a much less extent than O⁶-alkylguanines, but are also much less efficiently removed from DNA, serving in this way as good markers for cumulative DNA damage with alkylating agents. 3-alkyladenines are efficiently removed from DNA and excreted undegraded in the urine almost quantitatively, and therefore represent suitable markers for the non-invasive evaluation of acute effects of human exposure to alkylating agents.

Increased oxidative DNA damage is implicated in a wide field of inflammatory processes. DNA damage is induced by exogenous or endogenous oxidative stress (ROS) or nitrogen species (RNS) or metabolites of various chemical compounds like vinyl halides. In the wide spectrum of oxidative DNA-lesions the mutagenic adducts 1,N⁶-ethenodeoxyadenosine and 8-oxo-2-deoxyguanosine are generated to a high extent, thus serving as good markers for oxidative damage processes.

LT: Molecular biology of mutagens and carcinogens: B. Singer & D. Grunberger; Plenum Press, New York (1983) • Distribution of methyl and ethyl adducts following alkylation with monofunctional alkylating agents: D. T. Beranek; *Mutat. Res.* **231** (1), 11 (1990) • Lipid peroxidation as a potential endogenous source for the formation of exocyclic DNA adducts: F.L. Chung, et al.; *Carcinogenesis* **17**, 2105 (1996) • Monoclonal antibody-based quantification and repair analysis of specific alkylation products in DNA: J. Thomale, et al.; Technologies for detection of DNA damage and mutations, G.P. Pfeiffer (ed), Plenum Press, New York (1996) • DNA repair and cellular resistance to alkylating agents in chronic lymphocytic leukemia: M. R. Müller, et al., *Clin. Cancer Res.* **3**, 2055 (1997) • Nucleic acid sequence and repair: role of adduct, neighbor bases and enzyme specificity: B. Singer & B. Hang; *Carcinogenesis* **21**, 1071 (2000) • Smoking related DNA and protein adducts in human tissues: D. H. Phillips; *Carcinogenesis* **23**, 1979 (2002) • Mechanisms of human DNA repair: an update: M. Christmann, et al., *Toxicology* **193** (1-2); 3 (2003) • Oxidative DNA damage and disease: induction, repair and significance: M. D. Evans, et al., *Mutat. Res. Rev.* **567**, 1 (2004)

Method	Sensitivity	Detection limit ^a (O ⁶ -EtG/Guo in DNA)	Specificity in samples with known/unknown exposure	Some typical applications
HPLC-RIA	50 fmol	1 x 10 ⁻⁷	High/High	Molecular epidemiology; quantification of absolute damage concentrations
ISB	1 fmol	1 x 10 ⁻⁷	High/Moderate ^b	Screening of DNA samples (epidemiological studies); analysis of formation and repair in experimental cell systems
IA-PCR	-	0.5 x 10 ⁻⁷	High/Moderate ^b	Formation of specific DNA lesions and their repair in known DNA sequences; screening for "hotspots"
IHC	1 x 10 ⁻²¹ mol	1 x 10 ⁻⁷	High/Moderate ^b	Analysis of DNA adduct formation and repair in specific cell types, tissue sections and in small bioptical cell samples

- a. Calculation of the detection limit is based on sample sizes of (1) HPLC-RIA, 1 mg DNA; (2) ISB, 5 µg DNA; (3) IA-PCR, 5 µg DNA; (4) IHC, 100 cells.
b. The selectivity of the assay is based on antibody binding; this restricts the specificity if structurally similar, crossreactive DNA modifications are present in the samples.

Abbreviations:

RIA: Competitive Radioimmunoassay

ISB: Immuno Slot Blot Assay

IA-PCR: Immunoaffinity-PCR

IHC: Immunohistochemistry

FIGURE 1: Comparison of different immunoanalytical quantification methods for DNA alkylation products (values for O⁶-Ethyl-2-deoxyguanosine (O⁶-EtG) are presented for comparison).

highlight

Detection of O-alkylation

MAB to 3-Ethyladenine (EM 6-47)

SQX-SQM013.1 50 µg

CLONE: EM 6-47. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** 3-Ethyladenine. **SPECIFICITY:** Recognizes human, mouse and rat 3-ethyladenine. **APPLICATION:** ELISA, RIA.

LIT: Monoclonal antibodies for the specific detection of 3-alkyladenines in nucleic acids and body fluids: G. Eberle, et al.; *Carcinogenesis* **11**, 1753 (1990) • Tautomer-specific anti-(N-3 substituted)-adenine antibodies. New tools in molecular dosimetry and epidemiology: K. H. Glüsenkamp, et al.; *Angew. Chem. Int. Ed. Engl.* **32**, 1640 (1993)

3-Ethyladenine (MAB EM 6-47) Hydra® Immunoaffinity Support

SQX-SQCM013 1 ml column

MAB to 3-Ethyladenine (EM 6-47) coupled to 1 ml Hydra® support for immunopurification of 3-ethyladenine from biological samples. **DNA CAPACITY:** Approx. $1-3 \times 10^{-9}$ Mol.

LIT: Urinary excretion of 3-methyladenine and 3-ethyladenine after controlled exposure to tobacco smoke: A. Kopplin, et al.; *Carcinogenesis* **16**, 2637 (1995)

MAB to O⁶-Ethyl-2-deoxyguanosine (EM 21)

SQX-SQM004.1 100 µg

CLONE: EM 21. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** O⁶-Ethyl-2-deoxyguanosine. **SPECIFICITY:** Recognizes human, mouse and rat O⁶-methyl- and ethyl-2-deoxyguanosine. **APPLICATION:** ELISA, ISB, RIA.

LIT: Glial cell-specific differences in repair of O⁶-methylguanine: S.P. LeDoux, et al.; *Cancer Res.* **56**, 5615 (1996) • Determination of N⁷- and O⁶-methylguanine in rat liver DNA after oral exposure to hydrazine by use of immunochemical and electrochemical detection methods: J.H. van Delft, et al.; *Fundam. Appl. Toxicol.* **35**, 131 (1997) • Mutagenicities of N-nitrosodimethylamine and N-nitrosodiethylamine in *Drosophila* and their relationship to the levels of O-alkyl adducts in DNA: Y. Goto, et al.; *Mutat. Res.* **425**, 125 (1999)

O⁶-Ethyl-2-deoxyguanosine (MAB EM 21) Hydra® Immunoaffinity Support

SQX-SQCM004 1 ml column

MAB to O⁶-Ethyl-2-deoxyguanosine (EM 21) coupled to 1 ml Hydra® support for immunopurification of O⁶-ethyl-2-deoxyguanosine from biological samples. **DNA-CAPACITY:** Approx. $1-3 \times 10^{-9}$ Mol.

MAB to O⁶-Ethyl-2-deoxyguanosine (ER 6)

SQX-SQM001.1 50 µg

CLONE: ER 6. **ISOTYPE:** Rat IgG2b. **IMMUNOGEN:** O⁶-Ethyl-2-deoxyguanosine. **SPECIFICITY:** Recognizes human, mouse and rat O⁶-methyl- and ethyl-2-deoxyguanosine. **APPLICATION:** ELISA, IHC, IA-PCR, ISB, RIA.

LIT: Binding and repair of O⁶-ethylguanine in double-stranded oligodeoxynucleotides by recombinant human O⁶-alkylguanine-DNA alkyltransferase do not exhibit significant dependence on sequence context: K. Bender, et al.; *Nucl. Acids Res.* **24**, 2087 (1996) • Formation and persistence of O⁶-ethylguanine in genomic and transgene DNA in liver and brain of lambda(lacZ) transgenic mice treated with N-ethyl-N-nitrosourea: E.J. Mientjes, et al.; *Carcinogenesis* **17**, 2449 (1996) • Fast repair of O⁶-ethylguanine, but not O⁶-methylguanine, in transcribed genes prevents mutation of H-ras in rat mammary tumorigenesis induced by ethylnitrosourea in place of methylnitrosourea: J. Engelbergs, et al.; *PNAS* **95**, 1635 (1998) • Mutagenicities of N-nitrosodimethylamine and N-nitrosodiethylamine in *Drosophila* and their relationship to the levels of O-alkyl adducts in DNA: Y. Goto, et al.; *Mutat. Res.* **425**, 125 (1999)

O⁶-Ethyl-2-deoxyguanosine (MAB ER 6) Hydra® Immunoaffinity Support

SQX-SQCM001 1 ml column

MAB to O⁶-Ethyl-2-deoxyguanosine (ER 6) coupled to 1 ml Hydra® support for immunopurification of O⁶-ethyl-2-deoxyguanosine from biological samples. **DNA-CAPACITY:** Approx. $1-3 \times 10^{-9}$ Mol.

MAB to O⁶-Ethyl-2-deoxyguanosine (ER 17)

SQX-SQM002.1 50 µg

CLONE: ER 17. **ISOTYPE:** Rat IgM. **IMMUNOGEN:** O⁶-Ethyl-2-deoxyguanosine. **SPECIFICITY:** Recognizes human, mouse and rat O⁶-ethyl-2-deoxyguanosine. **APPLICATION:** ELISA, IHC, RIA.

LIT: Quantification of specific DNA O-alkylation products in individual cells by monoclonal antibodies and digital imaging of intensified nuclear fluorescence: F. Seiler, et al.; *Carcinogenesis* **14**, 1907 (1993) • Repair of O⁶-alkylguanines in the nuclear DNA of human lymphocytes and leukaemic cells: analysis at the single-cell level: J. Thomale, et al.; *Br. J. Cancer* **69**, 698 (1994)

O⁶-Ethyl-2-deoxyguanosine (MAB ER 17) Hydra® Immunoaffinity Support

SQX-SQCM002 1 ml column

MAB to O⁶-Ethyl-2-deoxyguanosine (ER 17) coupled to 1 ml Hydra® support for immunopurification of O⁶-ethyl-2-deoxyguanosine from biological samples. **DNA-CAPACITY:** Approx. $1-3 \times 10^{-9}$ Mol.

MAB to O²-Ethyl-2-deoxythymidine (EM 4-1)

SQX-SQM015.1 200 µg

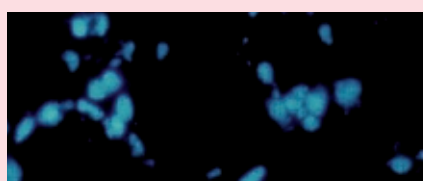
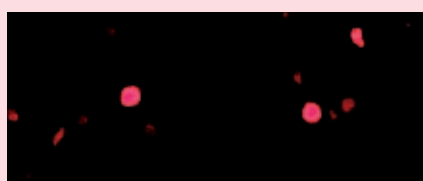
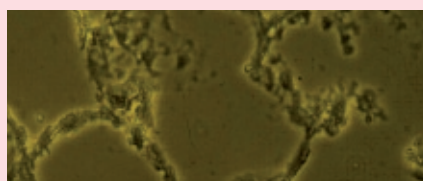
CLONE: EM 4-1. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** O²-Ethyl-2-deoxythymidine. **SPECIFICITY:** Recognizes human, mouse and rat O²-ethyl-2-deoxythymidine. **APPLICATION:** ICC, RIA.

MAB to O⁶-Methyl-2-deoxyguanosine (EM 2-3)

SQX-SQM003.1 100 µg

CLONE: EM 2-3. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** O⁶-Methyl-2-deoxyguanosine. **SPECIFICITY:** Recognizes human, mouse, rat and hamster O⁶-methyl-, ethyl- and butyl-2-deoxyguanosine. **APPLICATION:** ELISA, IHC, IA-PCR, RIA.

LIT: Formation and persistence of the miscoding DNA alkylation product O⁶-ethylguanine in male germ cells of the hamster: F. Seiler, et al.; *Mutat. Res.* **385**, 205 (1997) • Fast repair of O⁶-ethylguanine, but not O⁶-methylguanine, in transcribed genes prevents mutation of H-ras in rat mammary tumorigenesis induced by ethylnitrosourea in place of methylnitrosourea: J. Engelbergs, et al.; *PNAS* **95**, 1635 (1998)



O⁶-Methyl-2-deoxyguanosine (MAB EM 2-3) Hydra® Immunoaffinity Support

SQX-SQCM003 1 ml column

MAB to O⁶-Methyl-2-deoxyguanosine (EM 2-3) coupled to 1 ml Hydra® support for immunopurification of O⁶-methyl-2-deoxyguanosine from biological samples. **DNA-CAPACITY:** Approx. $1-3 \times 10^{-9}$ Mol.

FIGURE: Pneumocyte type II cell specific formation of O⁶-ethyl-2-deoxy-guanosine adducts in the nuclear DNA of lung tissue cells from rats 6 h after exposure to diethylnitrosamine (100 mg/kg b.w.). IHC-analysis of cryosections using adduct-specific EM 2-3 antibodies (red) (SQX-SQM003.1) and DNA counterstaining with DAPI (blue).

Detection of Oxidative DNA Lesions

MAb to 1,N⁶-Etheno-2-deoxyadenosine (EM A-1)

SQX-SQM009.1

50 µg

CLONE: EM A-1. ISOTYPE: Mouse IgG2a. IMMUNOGEN: 1,N⁶-Etheno-2-deoxyadenosine. SPECIFICITY: Recognizes human and rat 1,N⁶-etheno-2-deoxyadenosine. APPLICATION: IHC, RIA.

LIT: Immunohistochemical detection of 1,N⁶-ethenodeoxyadenosine in nuclei of human liver affected by diseases predisposing to hepatocarcinogenesis: A. Frank, et al.; Carcinogenesis 25, 1027 (2004)

1,N⁶-Etheno-2-deoxyadenosine (MAb EM A-1) Hydra® Immunoaffinity Support

SQX-SQCM009

1 ml column

MAb to 1,N⁶-Etheno-2'-deoxyadenosine (EM A-1) coupled to 1 ml Hydra® support for immunopurification of 1,N⁶-etheno-2'-deoxyadenosine from biological samples. DNA-CAPACITY: Approx. 1-3 x 10⁻⁹ Mol.

MAb to 8-Oxo-2-deoxyguanosine (EM 8oxo-4)

SQX-SQM021.1

100 µg

CLONE: EM 8oxo-4. ISOTYPE: Mouse IgG1. IMMUNOGEN: 8-Oxo-2-deoxyguanosine. SPECIFICITY: Recognizes 8-oxo-2-deoxyguanosine. APPLICATION: ELISA, RIA.

8-Oxo-2-deoxyguanosine (MAb EM 8oxo-4) Hydra® Immunoaffinity Support

SQX-SQCM021

1 ml column

MAb to 8-Oxo-2-deoxyguanosine coupled to 1 ml Hydra® support for immunopurification of 8-oxo-2-deoxyguanosine from biological samples. DNA-CAPACITY: Approx. 1-3 x 10⁻⁹ Mol.

PAb to 8-Oxo-2-deoxyguanosine

SQX-SQP003.1

100 µg

From rabbit. SPECIFICITY: Recognizes rat 8-oxo-2-deoxyguanosine. APPLICATION: IHC, RIA.

LIT: Formation and persistence of 8-oxoguanine in rat lung cells as an important determinant for tumor formation following particle exposure: P. Nehls, et al.; Environ. Health Perspect. 105 Suppl 5, 1291 (1997)

8-Oxo-2-deoxyguanosine Hydra® Immunoaffinity Support

SQX-SQCP003

1 ml column

PAb to 8-Oxo-2-deoxyguanosine coupled to 1 ml Hydra® support for immunopurification of 8-oxo-2-deoxyguanosine from biological samples. DNA-CAPACITY: Approx. 1-3 x 10⁻⁹ Mol.

Performance of Various Methodologies for Immunoanalytical Detection of DNA Lesions

Prod. Name	Prod. No.	ELISA	IHC	RIA	ISB	IA-PCR
MAb to 1,N ⁶ -Etheno-2-deoxyadenosine (EM A-1)	SQX-SQM009.1	n.d.	+	+	n.d.	n.d.
MAb to 3-Ethyladenine (EM 6-47)	SQX-SQM013.1	+	-	+	n.d.	n.d.
MAb to O ⁶ -Ethyl-2-deoxyguanosine (ER 6)	SQX-SQM001.1	+	+	+	+	+
MAb to O ⁶ -Ethyl-2-deoxyguanosine (ER 17)	SQX-SQM002.1	+	+	+	n.d.	n.d.
MAb to O ⁶ -Ethyl-2-deoxyguanosine (EM 21)	SQX-SQM004.1	+	-	+	+	n.d.
MAb to O ² -Ethyl-2-deoxythymidine (EM 4-1)	SQX-SQM015.1	n.d.	ICC	+	n.d.	n.d.
MAb to O ⁶ -Methyl-2-deoxyguanosine (EM 2-3)	SQX-SQM003.1	+	+	+	n.d.	+
MAb to 8-Oxo-2-deoxyguanosine (EM 8oxo-4)	SQX-SQM021.1	+	n.d.	+	n.d.	n.d.
PAb to 8-Oxo-2-deoxyguanosine	SQX-SQP003.1	n.d.	+	+	n.d.	n.d.

Abbreviations:

ELISA: Enzyme Linked Immunosorbent Assay

IHC: Immunohistochemistry

RIA: Radioimmunoassay

ISB: Immuno Slot Blot Assay

IA-PCR: Immunoaffinity-PCR

ICC: Immunocytochemistry

Latest Additions

ImmunoSelect® Adhesion Slides

SQX-SQ-IS-10.050 50 Slides
SQX-SQ-IS-10.100 100 Slides

ImmunoSelect® Adhesion Slides have been developed for use with microscopes, onto which available cellular material should be efficiently immobilized. In contrast to commonly coated slides the ImmunoSelect® Adhesion Slides stop cell loss even under harsh incubation procedures. The ImmunoSelect® adhesion surface allows a fast and highly efficient immobilization of the cells and helps to reduce the amount of needed cellular material and reagents. The extremely fast binding of the cellular material to the glass surface saves time-consuming centrifugation and drying procedures.

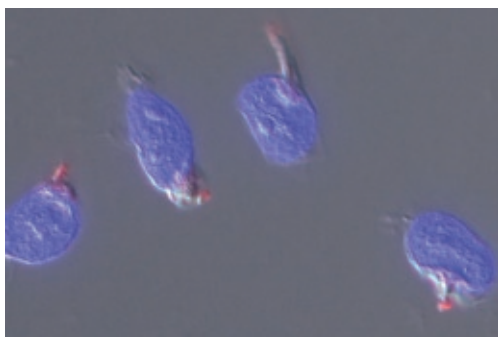


FIGURE: Polarized human CD34⁺ hematopoietic cells, adsorbed to ImmunoSelect® Adhesion Slides. DIC and immunohistological staining anti-Flo-1 (red), and DNA (DAPI, blue). J. Beckmann, Institute for Transplantation Diagnostics at the University of Düsseldorf, Germany.

Literature References:

Stringent regulation of DNA repair during human hematopoietic differentiation: a gene expression and functional analysis: T.U. Bracker, et al.; *Stem Cells* **24**, 722 (2006)

Adduct-specific monoclonal antibodies for the measurement of cisplatin-induced DNA lesions in individual cell nuclei: B. Liedert, et al.; *Nucleic Acids Res.* **34**, e47 (2006)

High accumulation of platinum-DNA adducts in strial marginal cells of the cochlea is an early event in cisplatin but not carboplatin ototoxicity: J.P. Thomas, et al.; *Mol. Pharmacol.* **70**, 23 (2006)

Application of Adhesion Slides

- Immunofluorescence methods or other comparable methods
- Immunoenzymatic tests (e.g. Peroxidase, Alkaline Phosphatase)
- Histological staining techniques (e.g. Pappenheim stain)
- Intracellular antigen evidencing
- Molecular biological tests (e.g. FISH or the detection of specific DNA modifications)

Compatibility with Staining Techniques

The adhesion slides are tested for several fluorescence dyes:

- Fluorescein derivatives (e.g. FITC)
- Rhodamine derivatives (e.g. TRITC, Texas Red)
- Cy3 and Cy5
- Phycobilliproteins (e.g. PE)
- DAPI
- Hoechst 3334 and 33358

YOUR ADVANTAGES!

- **Very fast adhesion of cells and tissue sections with high retention (>95%)!**
- **New alternative to polylysine and other adhesion techniques**
- **No cytopins or smears necessary, simply drop the cells and let them float down**
- **Cell adhesion resistant to heating, staining and denaturation procedures**
- **No cell loss even under harsh cytological staining procedures**
- **Superb retention of cell and tissue morphology**

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