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## Chemiluminescence ELISA for the Detection of Oxidative DNA Base Damage Using Anti-8-hydroxy-2'-deoxyguanosine Antibody : Application to the Detection of Irradiated Foods

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*Since ionizing radiation is used for sterilizing or lowering the microbial content of foods as a means of reducing food losses and securing food safety, the development of versatile detection methods of irradiated foods is necessary for appropriate management. In an effort to distinguish between irradiated and non-irradiated food, a method based on the detection of oxidative DNA base damage using the chemiluminescence enzyme-linked immunosorbent assay (ELISA) with anti-8-hydroxy-2'-deoxyguanosine antibody was developed. In the course of optimizing the reaction conditions for the ELISA, a 30-mer synthetic oligonucleotide containing 8-hydroxyguanine (8-oxoG) was used. Under the optimized conditions, the correlation between chemiluminescence intensity and 8-oxoG content in oligonucleotides was obtained. It was shown that this chemiluminescence ELISA method could be applied to chicken, beef and pork that were irradiated with over 3 kGy. Twenty milligrams of a loaf of meat was sufficient to distinguish between irradiated and non-irradiated meat by this method.*

Key Words : food irradiation, detection method, non-hydrolyzed DNA, enzyme-linked immunosorbent assay, oxidative DNA base damage, 8-hydroxyguanine, anti-8-hydroxy-2'-deoxyguanosine antibody, oligonucleotide, comet assay, chemiluminescence

### 1. Introduction

Ionizing radiation can cause a variety of physical, chemical and biological changes directly and indirectly including free radical induction, DNA damage, gene mutation and cell death<sup>1)</sup>. Using the lethal effects of radiation against pests and microbes, food irradiation is carried out for reasons of public health, the re-

duction of post-harvest losses and quarantine

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treatment of certain foods<sup>2</sup>). Each and every irradiated food should be noted in relevant shipping documents and then only be made available to consumers as products with clear labels indicating the use of irradiation and/or the international logo, as described in a Codex General Standard<sup>3</sup>). Informed consumers can then choose between irradiated or un-irradiated foods following enforcement of labeling rules. Detection methods for irradiated foods are required to endorse the reliability of labels. To date, several detection methods have been developed. For example, gas chromatography-mass spectrometry (GC-MS), electron spin resonance (ESR) spectroscopy and thermoluminescence (TL) analysis have essentially been used for the detection of 2-alkylcyclobutanone<sup>4</sup>), trapped free radicals<sup>5</sup>), and light emission from thermal re-annealing of trapped electrons or positive holes<sup>6</sup>) caused by radiation, respectively. However, there are difficulties concerning implementation of the aforementioned methods. GC-MS needs to be carried out by well-trained people since trace products derived from lipids have to be detected. ESR spectroscopic equipment is prohibitively expensive for most laboratories, while TL analysis requires an irradiation device since TL intensities emitted from samples irradiated with uncertain doses need to be normalized with a fixed dose. Furthermore, each of the aforementioned detection methods possesses limited validity for the kind of irradiated foods usually examined. Therefore, a new detection method is required that can expand the range of available irradiated foods to be examined.

Radiation-induced DNA damage can be used as a marker for the detection of irradiated food. The comet assay, which can detect DNA strand breaks, was adopted as a screening test

in the European Committee for Standardization<sup>7</sup>), but the outcome of the assay was affected by the variation in food storage temperature and period. GC-MS can also be used to detect radiation-modified bases including 2,6-diamino-4-hydroxy-5-formamidopyrimidine, 8-hydroxyguanine (8-oxoG), 5-hydroxyhydantoin, thymine glycol and 4,6-diamino-5-formamidopyrimidine<sup>8</sup>). Recently, the enzyme-linked immunosorbent assay (ELISA) has been used to quantify the amount of 8-hydroxy-2'-deoxyguanosine (8-OHdG), a deoxyribonucleoside possessing an 8-oxoG base, in urine or blood serum. This technique can detect 8-OHdG in DNA following hydrolysis of the DNA with nuclease and alkaline phosphatase to yield mononucleosides.

Two 8-OHdG detection kits utilizing the ELISA with different measuring ranges are commercially available. Use of the kits involves three steps: (i) competitive reaction of primary antibody between prefixed antigen on a microplate and a free one in the sample, (ii) additional reaction with secondary antibody linked to horseradish peroxidase against the primary antibody, and (iii) reaction with a substrate of the linked enzyme. Quantitative estimation from measurements is achieved through use of a calibration curve. The anti-8-OHdG antibody used in these kits is specific to 8-OHdG<sup>9</sup>). No cross-reactivity is shown to analogs of 8-OHdG, except for 8-sulfhydrylguanosine and 8-hydroxyguanosine (RNA type) with values less than 1%, indicating that the epitope spreads from the hydroxyl group (C8) of guanine to the deoxy site (C2') of 8-OHdG<sup>9</sup>). This antibody was also used for immunohistochemical staining of microscopic preparations<sup>9</sup>). The antibody was expected to recognize 8-OHdG between phosphodiester bonds in genomic DNA, even though the antibody was raised against mono-

nucleoside 8-OHdG<sup>6)</sup>.

Radiation-induced DNA damage generally results from OH radicals generated from the radiolysis of water<sup>1)</sup>. The production of OH radicals is also thought to be contributed by electron leaks from the respiratory chain<sup>10)</sup>. These considerations suggest that sensitive quantitative measurements are required to detect irradiated foods given the generation of spontaneous and radiation-induced 8-oxoG. The ELISA is typically employed for quantitative measurements. When employing the presently available detection kits for 8-OHdG, the DNA sample needs to be completely digested to yield nucleosides. If this DNA digestion step can be omitted, the process of detecting irradiated foods would be significantly simplified due to the reduction in sample handling and the need for additional experimental equipment. In this report, a novel and easy detection method is proposed to identify irradiated foods by detecting 8-oxoG that commonly exists in irradiated foods. For practical application purposes, we examined 8-oxoG in irradiated meat using the ELISA.

## 2. Experimental

### 2.1 Synthetic oligonucleotides

Synthetic oligonucleotides shown in Fig. 1 were purchased from Sigma Genosys (Hokkaido, Japan), except for damaged DNA containing 8-oxoG (Tsukuba Oligo Service, Ibaraki, Japan).

### 2.2 $\gamma$ irradiation

Ground meat of pigs, chicken and cattle grown domestically were purchased from a market in Japan. Meat samples were placed into polyethylene bags and then formed into a rod shape of about 1-cm diameter. Requisite samples were placed on ice and then irradiated

with  $\gamma$  rays of <sup>60</sup>Co at a dose rate of 3 kGy/h at the Takasaki Advanced Radiation Research Institute in Japan Atomic Energy Agency Takasaki, Japan). Following  $\gamma$  irradiation, samples were kept in a freezer at -20 °C until DNA isolation.

### 2.3 Preparation of genomic DNA from meat samples

A DNeasy Tissue Kit (Qiagen, Hilden, Germany) was used to isolate genomic DNA from the various meat samples, producing the high-molecular-weight DNA up to 50 000 base pairs (bp) in size, with fragments of 30 kbp predominating. A principle of DNA isolation using the kit is based on selective adsorption of DNA onto the silica-gel membrane matrix in a column settled in a microcentrifuge tube. The procedure was performed according to the manufacturer's instructions with slight modification. Briefly, a portion of meat sample was mashed under liquid nitrogen using a mortar and pestle. Twenty milligrams of meat powder was suspended in 180  $\mu$ L ATL buffer of the kit and treated with RNase A (0.28 mg/mL) at 37 °C for 1 h. Subsequent to this, DNA was isolated according to the kit instructions. DNA was finally eluted in 200  $\mu$ L of sterilized distilled water and the concentration was determined from the absorbance at 260 nm (When  $A_{260} = 1.0$ , the concentration of DNA solution is calculated as 50  $\mu$ g/mL).

### 2.4 ELISA procedure

For all ELISA tests, Microlite 2+ (Thermo Electron Co., MA) microtiter plates pretreated with 1% protamine sulfate were used. Anti-8-OHdG monoclonal antibody (N45.1 ; Nikken SEIL Co., Shizuoka, Japan) was used as primary antibody to detect 8-oxoG. Peroxidase-la-

beled anti-mouse-IgG (H + L) antibody (Kirkegaard and Perry Laboratories, Inc., MD) (KPL) was used as the secondary antibody. For detection utilizing the ELISA, a chemiluminescent substrate kit (KPL) including LumiGLO Reserve and Washing Solution (20x concentrate) was used according to the manufacturer's instructions.

The solution containing oligonucleotide(s) or genomic DNA was heated in a boiling water bath for 10 min, chilled immediately in ice-cold water, mixed with both 1/10 volume of 10% BSA diluent/blocking solution (KPL) and 1/50 volume of primary antibody stock solution (0.1 mg/mL), and then placed into wells of the microtiter plate. The DNA-primary antibody mixtures were incubated for 3 h at room temperature (about 20 – 25°C). After washing five times with Washing Solution (1x), the plate was blocked with 360  $\mu$ L of 1% BSA diluent/blocking solution for 1 h at room temperature, and then incubated with 100  $\mu$ L of secondary antibody containing 0.25  $\mu$ g/mL in 1% BSA diluent/blocking solution for 1 h. The plate was then washed five times with Washing Solution (1x). One hundred microliters of LumiGLO Reserve (KPL) was then added to the wells to initiate peroxidase activity. The chemiluminescence intensity was then immediately measured using the Lumi-Imager F1 Workstation (Roche, Basel, Switzerland) and analyzed using LumiAnalyst software (Roche).

### 3. Results and Discussion

#### 3.1 Anti-8-OHdG antibody could detect the 8-oxoG base present within oligonucleotides

Anti-8-OHdG antibody was originally established to recognize mononucleoside 8-OHdG. Although anti-8-OHdG antibody is a mouse

monoclonal antibody raised against 8-OHdG as an antigen, the antibody has been used for immunohistochemical staining without DNA hydrolysis. In order to apply the antibody to the detection of irradiated food, the specificity of the antibody was determined against 8-OHdG, where the 2'-deoxyribose is located between phosphodiester bonds in DNA, using synthetic oligonucleotides as targets for the detection.

The primary antibody has been shown not to react with 2'-deoxyguanosine, 2'-deoxyadenosine, 2'-deoxycytidine and 2'-deoxythymidine<sup>9</sup>. Therefore, oligonucleotide CA30 was designed as the negative control to measure nonspecific binding, CA30(8-oxoG)15 as the positive control possessing a one-base replacement by 8-oxoG, CA30G15 as the sample to adjust for 8-oxoG density of the positive control sample, and TG 30C16 as the possible complementary sequence of CA30(8-oxoG)15 and CA30G15 (Fig. 1). The antibody was reacted with synthetic oligonucleotides in an effort to determine the specificity against an 8-oxoG in a single oligonucleotide. According to the difference between the chemiluminescence intensities of CA30 and CA 30(8-oxoG)15, it was apparent that the anti-8-OHdG antibody could recognize an 8-oxoG in the oligonucleotide (Fig. 2A). When the total amount of DNA is over 40 ng/well, saturation of chemiluminescence intensities was observed. To show the binding capability of the antibody against 8-oxoG in nucleotides, 30-mer oligonucleotides were used in this experiment. Since DNA isolated from foods initially consists of double-stranded sequences, the effect of the presence of complementary sequences in the reaction mixture was also examined. In the next experiment, the chemiluminescence intensity of 8-oxoG in the oligonucleotide was further examined below 7 ng/well (Fig. 2B).

- (A) CA30 5' -CACACACACACACACACACACACACA-3'
- (B) CA30G15 5' -CACACACACACACAGACACACACACACA-3'
- (C) CA30 (8-oxoG) 15 5' -CACACACACACAG\*ACACACACACACA-3'
- (D) TG30C16 5' -TGTGTGTGTGTGTCTGTGTGTGTGTG-3'

Fig. 1 Synthetic oligonucleotides. (A) CA30. This is a 30-mer deoxyribonucleotide possessing only cytosine and adenine bases. The oligonucleotide in itself keeps the single-stranded form. (B) CA30G15. This oligonucleotide replaced cytosine with guanine of CA30 at the 15th position from the 5'-end. (C) CA30(8-oxoG)15. An 8-hydroxyguanine indicated as G\* was used for the synthesis of this nucleotide sequence. The damage density was expected as 90 kGy-irradiated DNA equivalents at the point of 8-oxoG. (D) TG30C16. This is regarded as a possible complementary sequence for CA30G15 and CA30(8-oxoG)15. Since this oligonucleotide represents the construct of a repeating dimeric sequence, pairing with 8-oxoG might occur at various positions of the complementary sequence following renaturation.

In Fig. 2B, oligonucleotides CA30G15 and CA30(8-oxoG)15 were mixed with TG30C16, subjected to heat denaturation, cooled on ice, and then analyzed with the ELISA to measure the reactivity of the primary antibody in the presence of potential complimentary sequences. The chemiluminescence intensity of the ELISA against oligonucleotides increased linearly with

increasing 8-oxoG content, indicating that the primary antibody could react with the loci of the 8-oxoG base present within oligonucleotides. Fig. 2B showed that the oxidative DNA base damage is detected with linear response using this protocol, as described in Materials and Methods.

In establishing the ELISA, we chose to incubate the primary anti-

body and oligonucleotides for 3 h on protamine-coated microtiter plates. The 3 h incubation time was expected to be of sufficient duration for the plate to adsorb the nucleotide-primary antibody complexes with minimal oxidation of normal G bases.

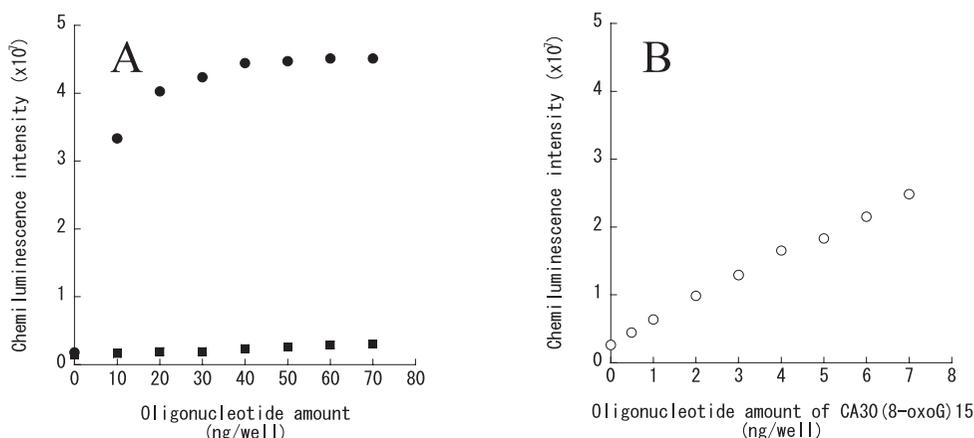


Fig. 2 ELISA detection of 8-oxoG. (A) Different amounts of single oligonucleotides. Symbols indicate ■, CA30 ; ●, CA30(8-oxoG)15. (B) Effect of mixed oligonucleotides. The ○ symbol indicates CA30G15 + CA30(8-oxoG)15 (total 20 ng/well of the two single nucleotides) mixed with TG30C16 (20 ng/well). Each point represents the mean value of quadruplicate measurements.

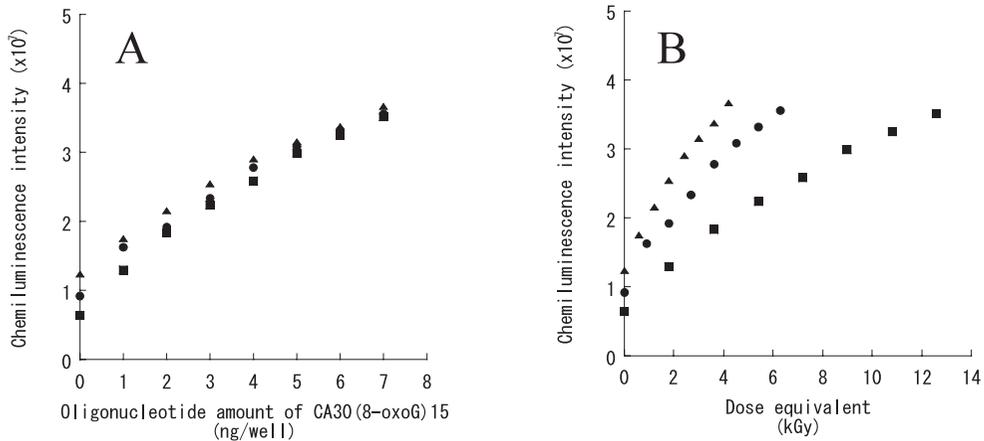


Fig. 3 Effect of oligonucleotide amount on the ELISA detection of 8-oxoG. (A) Relationship between total oligonucleotide amount and chemiluminescence intensity dependent on 8-oxoG. Half of the oligonucleotide applied in the microwell was TG30C16 while the remainder represented a mixture of CA30G15 and CA30(8-oxoG)15 of which the portion varied. Symbols indicate total DNA of 50 ng/well (■), 100 ng/well (●) and 150 ng/well (▲). (B) Relationship between dose equivalent and chemiluminescence intensity. Dose equivalent refers to calculated doses according to the density of 8-oxoG on the total oligonucleotide. Each point represents the mean value of quadruplicate measurements.

### 3·2 Chemiluminescence intensity of the ELISA corresponded to the 8-oxoG content of the oligonucleotide

In an effort to detect lower-dose irradiated samples, increasing the nucleotide amount in the microwell might be effective since this would increase the amount of damaged DNA present within the well. However, the chemiluminescence intensity originated from the primary antibody bound to 8-oxoG might be restricted at certain levels due to the limited adsorption capacity of oligonucleotides on the plate's surface. The effect of adsorption of oligonucleotides was therefore examined using the ELISA.

Different amounts of oligonucleotides were placed in microwells possessing variable 8-oxoG content from 0 to 7 ng, and the chemiluminescence intensity of 8-oxoG was measured. Fig. 3A shows that, although the 0-ng point of each curve differed slightly, only a slight vari-

ation in chemiluminescence intensity was observed in the 50 to 150 ng/well range according to the 8-oxoG content, indicating that oligonucleotides in the 50 to 150 ng/well range are quantitatively adsorbed onto the microtiter plates coated with protamine sulfate.

Nackerdian et al. measured the yield of 8-oxoG in  $\gamma$ -irradiated mammalian cells following by GC-MS<sup>8)</sup>. According to their results, 16 bases of 8-oxoG were detected out of 100 000 bases following 420-Gy irradiation. We estimated that one 8-oxoG out of 2 700 bases should be produced by 1-kGy irradiation. From the ratio of the 8-oxoG yield, our CA30(8-oxoG)15 oligonucleotide containing one 8-oxoG in 30 bases could be calculated to be equivalent to 90 kGy-irradiated DNA. When non-damaged CA30G15 and TG30C16 oligonucleotides were mixed with CA30(8-oxoG)15, we could arrange the density of 8-oxoG damage in unit quantity, meaning that mimicked irradiated oligonucleo-

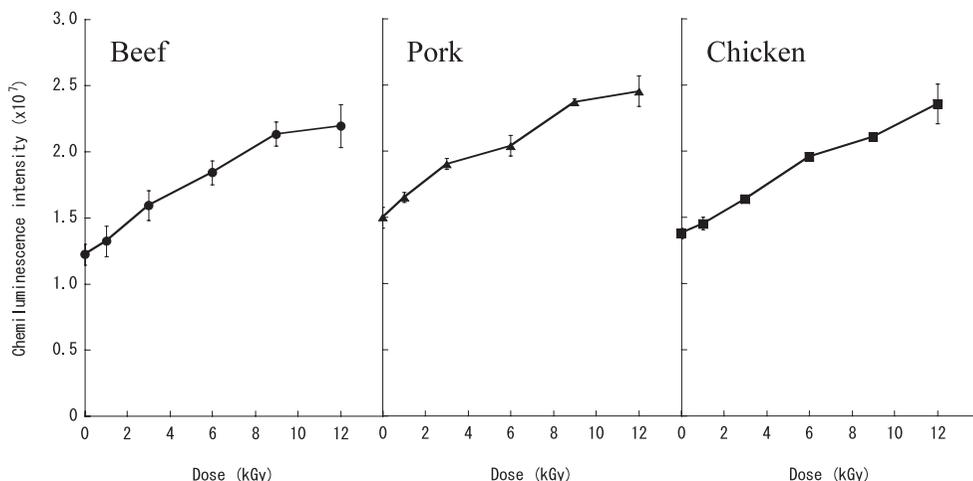


Fig. 4 ELISA detection against 8-oxoG DNA in irradiated meat. Symbols indicate DNA isolated from beef (●), pork (▲) and chicken(■). DNA at 100 ng/well was used for the ELISA. Each point represents the mean value and standard error from three independent experiments of different irradiation.

tides for the determination of 8-oxoG content could be prepared with certain dose equivalents. From this point of views, Fig. 3A was replotted as dose equivalent (Fig. 3B), indicating that a higher oligonucleotide content up to at least 150 ng/well yielded a steeper slope in terms of the quantitative ELISA.

### 3.3 The 8-oxoG within DNA of irradiated meat was detected using the novel ELISA method and showed a dose-dependency

To determine the practical application of our method on actual foods, ground chicken, beef and pork meat were irradiated on ice. For demonstration purposes, ground meat stored at -20 °C for 1 month following  $\gamma$  irradiation was examined. The chemiluminescence intensity of the ELISA was measured using 100 ng/well of DNA isolated from irradiated meat (Fig. 4). The results showed that the intensity increased with increasing doses. A linear dose-intensity response for irradiated beef was ob-

served up to 9 kGy, while that for chicken meat seemed to be within 6 kGy, and that for pork might be up to 3 kGy.

In living cells, spontaneous 8-oxoG lesions occur on DNA due to OH radicals that are produced by electron leaking from the electron transport system associated with endogenous metabolism<sup>10</sup>. This is thought to account for the basal content of 8-oxoG detected in non-irradiated meat. This fact obliged us to delineate the significant increment in the amount of 8-oxoG of non-irradiated foods. Although simply dose-response curves of meats were obtained in this report, according to standard errors of means, it is thought that there is a possibility to distinguish meat irradiated with approximately 3 kGy or more from non-irradiated meat.

Even after 1-year storage at -20 °C following irradiation, our detection method was capable of showing the dose dependency of the intensity related to 8-oxoG content in the meat samples (data not shown). Although 8-oxoGs on DNA can be eliminated by DNA glycosylase as

in living cells and may be spontaneously released through depurination reaction, the release of 8-oxoGs would be seldom occurred in cells of meat, especially under the frozen condition. Therefore, this method can accurately detect 8-oxoG following long periods of storage. It is believed that this method has potential as an alternative to the comet assay. In the comet assay, DNA single- or double-strand breaks can be evaluated. Generally ice crystals formed in cells during repetitive freezing and thawing of meat must induce DNA breaks, in addition to cell membrane damage<sup>11), 12)</sup>. Therefore, most indicative values such as the tail length or the tail moment of the comet assay may change depending on the storage conditions of the food<sup>13)</sup>. The comet assay encounters true difficulty in relation to the preparation of a non-irradiated control possessing the corresponding storage history as the samples to be tested. In the case of our novel method, since 8-oxoG can be employed as the marker for oxidative DNA base damage, the production of 8-oxoG in food would not be influenced following standard storage conditions.

In the detection of hydrocarbons and 2-alkylcyclobutanones as the marker of irradiated food, about 10 g of meat sample is needed to extract radiolytic products of fat. According to our experiments, at least 1.8  $\mu$ g DNA could be recovered from 20 mg meat using DNeasy Tissue Kit. Since we apply 100 ng DNA per well of the microtiter plate, 20 mg as a meat sample is sufficient amount to examine the 8-oxoG content of DNA by ELISA.

#### 4. Conclusion

Anti-8-OHdG antibody was shown to bind to 8-oxoG loci on DNA without the prerequisite for DNA enzymatic digestion to produce mono-

nucleosides from high molecular weight genomic DNA. To be carried out simply using commercially available reagents and kits, a novel method utilizing anti-8-OHdG antibody was developed with optimization for detecting irradiated foods by 8-oxoG as a marker in DNA. The procedure is straightforward and can be executed in a relatively short time by technicians without the need for specialized training. This procedure can be applied to most foods that contain DNA, perhaps including grains and fruits to be examined in future. In the case of meat, 20 mg sample was sufficient for the 8-oxoG detection, and 3 kGy- or higher-irradiated meats were expected to be distinguished from non-irradiated samples.

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#### References

- 1) von Sonntag, C., *The Chemical Basis of Radiation Biology*, Taylor & Francis, London (1987)
- 2) STI/PUB/788 Acceptance, Control of and Trade in Irradiated Food, pp.135-176, International Atomic Energy Agency, Vienna, Australia (1989)
- 3) CODEX STAN 106-1983, REV. 1-2003 Codex General Standard for Irradiated Foods, Codex Alimentarius Commission, Rome, Italy (2003)
- 4) Stevenson, M. H., Crone, A. V. J. and Hamilton, J. T. G., Irradiation detection, *Nature*, **344**, 202-203 (1990)
- 5) Desrosiers, M. F. and Simic, M. G., Postirradiation dosimetry of meat by electron spin resonance spectroscopy of bones, *J. Agric. Food Chem.*, **36**, 601-603 (1988)
- 6) Moriarty, T. F., Oduko, J. M. and Spyrou, N. M., Thermoluminescence in irradiated foodstuffs, *Nature*, **332**, 22 (1988)

- 7) EN13784 Foodstuffs—DNA comet assay for the detection of irradiated foodstuffs—Screening method, European Committee for Standardization, Brussels, Belgium (2001)
- 8) Nackerdien, Z., Olinski, R. and Dizdaroglu, M., DNA base damage in chromatin of  $\gamma$ -irradiated cultured human cells, *Free Radic. Res. Commun.*, **16**, 259-279 (1992)
- 9) Toyokuni, S., Tanaka, T., Hattori, Y., Nishiyama, Y., Yoshida, A., Uchida, K., Hiai, H., Ochi, H. and Osawa, T., Quantitative immunohistochemical determination of 8-hydroxy-2'-deoxyguanosine by a monoclonal antibody N45.1 ; Its application to ferric nitrilotriacetate-induced renal carcinogenesis model, *Lab. Invest.*, **76**, 365-374 (1997)
- 10) Wei, Y. H., Oxidative stress and mitochondrial DNA mutations in human aging, *Proc. Soc. Exp. Biol. Med.*, **217**, 53-63 (1998)
- 11) Alur, M. D. and Grecz, N., Mechanism of injury of *Escherichia coli* by freezing and thawing, *Biochem. Biophys. Res. Commun.*, **62**, 308-312 (1975)
- 12) Grecz, N., Hammer, T. L., Robnett, C. J. and Long, M. D., Freezing-thaw injury : Evidence for double strand breaks in *Escherichia coli* DNA. *Biochem. Biophys. Res. Commun.*, **93**, 1110-1113 (1980)
- 13) Faullimel, C., Ennahar, S., Aoude-Werner, D., Guterl, P. and Marchioni, E., DNA comet assay for the detection of time-temperature abuse during the storage of poultry, *J. Food Prot.*, **68**, 1414-1420 (2005)
- 14) IAEA, Clearance of Irradiated Food Database, [http : // nucleus.iaea.org/NUCLEUS/nucleus/Content/Applications/FICdb/FoodIrradiationClearances.jsp?module=cif](http://nucleus.iaea.org/NUCLEUS/nucleus/Content/Applications/FICdb/FoodIrradiationClearances.jsp?module=cif)

## 要 旨

### 抗 8-hydroxy-2'-deoxyguanosine 抗体を用いた DNA 酸化損傷検出のための 化学発光 ELISA 法 : 照射食品検知法への応用

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食糧の保存中の損耗防止や衛生確保, 食中毒防止のために電離放射線を食品に照射する場合, その適切な管理には汎用性のある照射食品検知法 (照射の有無の判別法) が必要である。そこで, 照射食品と非照射食品を識別するため, 抗 8-OHdG 抗体を用いた化学発光—酵素免疫測定 (ELISA) 法による DNA の酸化的塩基損傷検出法を開発した。ELISA 反応の条件検討は, 8-hydroxyguanine (8-oxoG) を含む 30-mer のオリゴヌクレオチドを用いて行い, その条件でオリゴヌクレオチド中の 8-oxoG 含量と化学発光強度の関係が得られた。この化学発光 ELISA 法では, 3 kGy を超える吸収線量で照射された鶏肉・豚肉・牛肉を識別できることが示唆された。この方法では, 照射肉と非照射肉の識別に要する肉片は 20 mg で十分である。