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ProductInformation

Ogg1 Assay Kit

Product Code **CS0710**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Ogg1 (8-oxoguanine-DNA glycosylase) is a DNA repair protein involved in the repair of the major product of DNA oxidation, the miscoding base 8-oxoguanine (8-oxo-G). Ogg1 is implicated in the first step of the base-excision repair process, removal of the 8-oxo-G damaged base from the DNA duplex, resulting in the creation of an apurinic/apyrimidinic (AP) site. Due to the slow AP-lyase reaction kinetics of Ogg1, the next step of *in vivo* AP-site removal may be aided by AP endonuclease (APE1).[1-3] Polymorphism in the human *OGG1* gene is associated with the risk of various cancers such as lung and prostate cancer.[4]

The Ogg1 Assay Kit is designed for Ogg1 activity detection in cells lysates or for assaying purified enzyme preparations. In addition, the Ogg1 control enzyme provided with the kit, enables screening of Ogg1 modulators. The Ogg1 activity assay is based on glycosylase activity that recognizes and removes the mutated base (8-oxo-G), which is followed by lyase activity that cleaves the AP strand of double-stranded DNA. The substrate is a double-stranded 23 base oligonucleotide. One strand contains 8-oxo-dG as the eleventh base and is radiolabeled with ^{32}P at its 5' end. It is annealed to its complementary strand (containing dC as the base opposite the 8-oxo-dG) to form the double-stranded 23 base oligonucleotide substrate. Ogg1 cleavage of the substrate results in a labeled 10-mer fragment and a non-labeled 13-mer fragment. Putrescine is added at the end of the reaction to facilitate strand cleavage.[5] This results in single-stranded oligonucleotide products, that are separated on a denaturing polyacrylamide gel. The ^{32}P labeled bands, detected by autoradiography, correspond to the cleaved 10-mer fragment and the original 23 base oligonucleotide strand.

The kit was tested on HEK-293T, BHK, A-431, BAEC, HFF, HepG2, and HeLa cell lines.

Components

The kit contains reagents sufficient for 180 reactions.

- Ogg1 Substrate, 8-oxo-G strand — 1 vial
  (Product Code O 4639)
  23-mer oligonucleotide, 200 pmole
  CTCTCCCTTC[OXOG]CTCCTTTCCTCT
- Ogg1 Substrate, complementary strand — 1 vial
  (Product Code O 4764)
  23-mer oligonucleotide, 260 pmole
  AGAGGAAAGGAGCGAAGGGAGAG
- Reaction Buffer 10X — 1.5 ml
  (Product Code R 7277)
  500 mM HEPES, pH 7.5, with 500 mM KCl, 0.25% TRITON™ X-100, and 10 µg/ml BSA
- Stop Solution — 1 ml
  (Product Code S 1568)
  90% Formamide, 0.1% w/v Bromophenol blue, 0.1% Xylene cyanole FF, and 20 mM EDTA
- Ogg1 (control enzyme) — 10 µg
  from mouse, recombinant,
  expressed in *E. coli*
  (Product Code O 2135)
  100 µg/ml
- Putrescine — 1 ml
  (Product Code P 6872)
  0.5 M, pH 8.0
- SigmaSpin Post-Reaction Purification Columns (Product Code S 5059) — 2 each

Equipment and Reagents Required but Not Provided

- Molecular biology grade water (Product Code W 4502) or autoclaved ultrapure (17 MΩ·cm or equivalent) water
- Microcentrifuge tubes, 1.5 ml – autoclaved
- γ-^{32}P- ATP, 10 mCi/ml
- T4 polynucleotide kinase (PNK) enzyme (Product Code P 4390)
- 10X PNK Buffer: 50 mM Tris-HCl, pH 7.9, with 10 mM $MgCl_2$, 1 mM DTT, and 100 mM NaCl
- Tris-Borate-EDTA buffer (TBE, Product Code T 9525)
- 15% polyacrylamide, denaturing (7 M urea) PAGE gel[6] and gel electrophoresis apparatus
- Microcapillary round tips (Product Code T 1906) (optional)
- Autoradiography film
- Automatic processor for autoradiography film or supplies for manual processing

Additional Reagents Required for Ogg1 Activity Assay with cell lysates

- Lysis buffer - CelLytic™ M Cell Lysis Reagent (Product Code C 2978) is recommended for cell extraction.
- Phenol–chloroform–isoamyl alcohol (25:24:1) reagent (Product Code P 3803)
- Transfer RNA, tRNA (Product Code R 9001)
- 3 M Sodium Acetate, pH 5.2 (Product Code S 7899)
- Ethanol ~100% and 90% cold solutions

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The product is shipped on wet ice and it is recommended to store the kit components at –20 °C. Upon arrival, the SigmaSpin Post-Reaction Purification Columns may be stored at 2–8 °C.

Preparation Instructions

It is recommended to use molecular biology grade water or autoclaved ultrapure (17 MΩ·cm or equivalent) water when preparing the reagents.

Cells lysate preparation - CelLytic™ M Cell Lysis Reagent (Product C 2978) is recommended for cell extraction. Another buffer suitable for the assay procedure is 50 mM Tris, pH 7.5, with 1 mM EDTA, 5% glycerol, and 50 mM NaCl. For other lysis buffers, it is advised to test the suitability of the buffer in the procedure using the positive control enzyme. Note that high concentrations of salts or cations may interfere with the procedure.

1X Reaction Buffer - Dilute an aliquot of Reaction Buffer 10X (Product Code R 7277) 10-fold with water. Eighty µl of 1x Reaction Buffer are sufficient for a minimum of 6 reactions using purified enzyme preparations (Procedure B).

Ogg1 Control Solution (5 µg/ml) - Dilute an aliquot of the 100 µg/ml Ogg1 control enzyme (Product Code O 2135) 20-fold with 1X Reaction buffer. Prepare fresh.

tRNA Solution (tRNA not supplied with the kit) - Prepare a solution of 1 mg/ml.

Denaturing Gel (not supplied with the kit)[6] - Prepare a 15% polyacrylamide denaturing gel containing 7 M urea and pre-run the gel for 30 minutes at 100 V.

Radiolabeled Substrate - Observe all regulations regarding handling of radioactive material. The ^{32}P radioactivity decays with time ($t_{1/2}$ = 14 days) and the prepared substrate may be used for up to 4 weeks. Note: The substrate components in this kit are sufficient to perform the labeling reaction twice, each reaction provides radiolabeled substrate sufficient for 90 Ogg1 activity assays.

1. Reconstitute the Ogg1 Substrate, 8-oxo-G strand (Product Code O 4639) with 24 µl of water. Store the remaining oligonucleotide solution at –20 °C.

2. Reconstitute the Ogg1 Substrate, complementary strand (Product Code O 4764) with 30 µl of water. Store the remaining oligonucleotide solution at –20 °C.

3. Warm one SigmaSpin column to room temperature.

4. Assemble the reagents required for substrate labeling (Table 1).

Table 1.
Reaction Scheme for Substrate Labeling

Reagent	Volume
10X PNK Buffer	3 µl
Ogg1 Substrate, 8-oxo-G solution	10 µl (100 pmole)
γ-^{32}P- ATP, 10 mCi/ml	3 µl (30 µCi)
T4 polynucleotide kinase (PNK) enzyme	1 µl
Water	13 µl
Total volume	30 µl

5. Incubate the substrate labeling mixture for 60 minutes at 37 °C.

6. Stop the reaction by incubating for 10 minutes at 70 °C.

7. Remove the free ATP from the labeled nucleotide using the SigmaSpin column. Invert the warmed column several times to suspend the matrix uniformly. Loosen the cap by a half turn and then snap off the bottom closure. Place the column into one of the collection tubes.

8. Centrifuge the column and the collection tube for 2 minutes at 700 x *g*. The column should appear semi-dry without any cracks. Discard the collection tube containing the equilibration buffer.

9. Place the column into a new collection tube. Carefully apply the labeling reaction mixture (step 6) to the center of the column so that the sample will not flow along the inner wall of the column.

10. Centrifuge at 700 x *g* for 4 minutes and collect the labeled oligonucleotide in the collection tube.

11. The labeled oligonucleotide is now ready for annealing to the Ogg1 Substrate, complementary strand. Add 13 µl of the complementary strand oligonucleotide solution (step 2) to the radiolabeled 8-oxo-G strand oligonucleotide solution (step 10).

12. Anneal the strands with the following incubations:
    1 minute at 95 °C,
    then 5 minutes at 37 °C,
    and finally 30 minutes at room temperature.

13. Store the labeled, double-stranded, 23 base oligonucleotide solution at –20 °C in a storage box suitable for radioactive material.

Reaction Mixture - Mix 10 µl of Reaction Buffer 10X with 2 µl of ^{32}P-labeled, double-stranded, 23 base oligonucleotide solution and 68 µl of water. Eighty µl of Reaction Mixture are sufficient for a minimum of 6 reactions using cell lysate (Procedure A) or 10 reactions with the Ogg1 purified enzyme (Procedure B).

Procedures

A. Ogg1 Assay Reaction with Cell Lysate Samples

1. Add X µl of cell lysate (80-350 µg of protein) or 2 µl of the Ogg1 Control Solution to a microcentrifuge tube according to the reaction scheme (Table 2).

Table 2.
Reaction Scheme for Cell Lysate Samples

	Cell Lysate	Ogg1 Control Solution	Ogg1 Modulator Solution	Lysis Buffer	Reaction Mixture
Test	X µl	---	---	88-X µl	12 µl
Blank	---	---	---	88 µl	12 µl
Modulator Reaction	X µl	---	Y µl	88-X-Y µl	12 µl
Positive Control	---	2 µl	---	86 µl	12 µl

Note: For reactions testing the effects of modulators, add up to 10 µl of the modulator solution to the appropriate tube. It may be necessary to incubate the modulator with the cell lysate prior to the addition of the substrate. Following this incubation, the Ogg1 activity may be determined.

2. Bring the final volume to 88 µl with lysis buffer.

3. Add 88 µl of lysis buffer to the Blank control tube.

4. Add 12 µl of the Reaction Mixture to each of the tubes. Mix by pipetting up and down, and then cap the tubes. Incubate for 60 minutes at 37 °C.

5. After the reaction is completed, the radiolabeled substrate and product must be extracted and precipitated. Add 100 µl of phenol–chloroform–isoamyl alcohol (25:24:1) reagent to each of the tubes and vortex vigorously for 10 seconds.

6. Centrifuge the mixture for 3 minutes at 14,000 x *g*.

7. Transfer the upper aqueous phase to a new microcentrifuge tube.

8. Re-extract the organic layer (phenol–chloroform–isoamyl alcohol) with the addition of 100 µl of water. Vortex vigorously for 10 seconds.

9. Centrifuge the mixture for 3 minutes at 14,000 x *g*.

10. Collect the upper aqueous phase and combine with the first aqueous phase (step 7). Discard the organic fraction.

11. Add 10 µl of 1 mg/ml tRNA Solution (10 µg) to the combined aqueous fractions to serve as a carrier.

12. Add 25 µl of 3 M Sodium Acetate, pH 5.2, and vortex.

13. Add 600 µl of cold absolute (~100%) ethanol and incubate at –20 °C for 30 minutes.

14. Centrifuge at 13,000 x *g* for 6 minutes at 4 °C and carefully aspirate the liquid.
Note: The pellet may be invisible.

15. Add 1 ml of cold 90% ethanol to the pellet.

16. Centrifuge at 13,000 x *g* for 6 minutes at 4 °C and carefully aspirate the liquid.
Note: The pellet may be invisible.

17. Invert the microcentrifuge tube on a filter paper for 10-15 minutes to dry the pellet.

18. Add 10 µl of water to the bottom of each tube and vortex.

19. Continue to section C.

B. Ogg1 Assay Reaction with Purified Enzyme Samples

1. Add 1-10 µl of a purified enzyme sample or 2 µl of the Ogg1 Control Solution to a microcentrifuge tube according to the reaction scheme (Table 3).

Table 3.
Reaction Scheme for Purified Enzyme Samples

	Enzyme Sample	**Ogg1 Control Solution**	**Ogg1 Modulator Solution**	**1x Reaction Buffer**	**Reaction Mixture**
Tested enzyme	X µl	---	---	12-X µl	8 µl
Blank	---	---	---	12 µl	8 µl
Modulator Reaction	---	2 µl	Y µl	10-Y µl	8 µl
Positive Control	---	2 µl	---	10 µl	8 µl

Note: For reactions testing the effects of modulators, add up to 10 µl of the modulator solution to the appropriate tube. It may be necessary to incubate the modulator with the purified enzyme sample prior to the addition of the substrate. Following this incubation, the Ogg1 activity may be determined.

2. Bring the final volume to 12 µl with 1x Reaction Buffer.

3. Add 12 µl of 1x Reaction Buffer to the Blank control tube.

4. Add 8 µl of the Reaction Mixture to each of the tubes. Mix by pipetting up and down, and then cap the tubes. Incubate for 60 minutes at 37 °C.

5. Continue to section C.

C. Electrophoresis on a Denatured Gel

1. Add 2.5 µl of Putrescine (Product Code P 6872) to each reaction and mix by vortexing.

2. Heat the reactions for 5 minutes at 95 °C.

3. Add 5 µl of Stop Solution (Product Code S 1568) to each reaction and mix by vortexing.

4. Heat the reactions for 5 minutes at 95 °C.

5. Keep the reactions on ice until loading on a gel.

6. Load 15-20 µl of each reaction on the gel using microcapillary round tips (optional).

7. Run the gel using Tris-Borate-EDTA running buffer at 100-200 V until the bromophenol blue dye (dark blue) migrates to 1 cm from the end of the gel.
   Note: Bromophenol blue and xylene cyanole FF run as approximately 10 and 41-base oligonucleotides, respectively.

8. Carefully disassemble the gel apparatus and lay the gel on a piece of clear, plastic wrap.

9. Cover the gel with another piece of clear, plastic wrap.
   Note: Do not let the gel dry, as it may crack.

10. Expose the gel to X-ray film for 1-3 hours at –70 °C. It is recommended to put two sheets of film on the gel in order to get at least one film properly exposed.

References

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