2015 10

Instructions for the 8-oxo-dG ELISA kit

Prepare the solutions and reagents listed below.

(Also listed in the protocol “Preparation of solutions and reagents”.)

8-oxo-dG standards in sterile PBS: From the stock solution labeled *8-oxo-dG 100 ng/ml* prepare 500 µl each of 6 different 8-oxo-dG concentrations (0.01, 0.1, 0.5, 1.0, 3.0, and 10 ng/ml) in sterile PBS to be used as standard for each plate. Should be prepared fresh for each experiment.

Primary Antibody (P-Ab) working solution (5.5 ml): The tube in the kit marked *Primary antibody* (stock solution) should be kept at -20 ˚C until the day of use. To prepare the P-Ab antibody working solution mix the content of the stock solution (20 µl) with 1 ml of sterile PBS. Transfer the solution to a 10-ml tube and add 4.5 ml of sterile PBS. Ca be stored at +4 ˚C for 2 weeks.

Secondary Antibody (S-Ab) working solution (15 ml): The tube in the kit marked *Secondary antibody* (stock solution 6.5 µl) should be kept at +4 ˚C until the day of use. To prepare the S-Ab working solution: add 1 ml of sterile PBS to the stock solution. Mix, transfer the solution to a 20-ml tube and add 14 ml of sterile PBS. Can be stored at +4 ˚C for two weeks.

Washing solution (242 ml): Mix the bottle marked *Washing solution* (22 ml) with 220 ml sterile PBS. Can be stored at +4 ˚C for two weeks.

Chromogen solution working solution: Add 12 ul H_2O_2 (30-37 %) to the tube marked *Chromogen solution.* Can be stored at +4 ˚C for two weeks.

TMB: ready to use

Staining Solution for 96 wells: Prepare from *Staining solution* provided in the kit by taking 16 ml of the solution and adding 25 µl TMB and 25 µl *Chromogen working* solution (with H_2O_2). Should be used within 1 hour after preparation. (Do not prepare more than you need.)

Materials and solutions needed for the ELISA but not included in the kit

Sterile PBS (1000 ml), ddH_2O, H_2O_2 (30-37 %) (12 µl), 1.5-ml Eppendorf tubes, 10 and 20-ml tubes, Two 8-channel pipettes and phosphoric acid (1.5 M, 15 ml, can be prepared in larger quantities and stored at room temperature for 4 weeks). Absorbance (450 nm) reader for 96-well plates.

Analysis of 8-oxo-dG in samples from serum or culture media that have been purified using Bond Elut column filtration

General: The protocol is designed for analysis of samples and standards in triplicate. All samples should be purified before analysis using the Bond Elut method described in “Sample preparation and filtration instructions”.
Keep samples and the 96-well plate on ice (+4˚C) at all times when not indicated otherwise.
Open the plastic pack with the 96-well plate just before use (if you do not use all the wells, store the remaining dry and at +4 ˚C). Label the wells to keep track of your samples/standards, see example at the end of instructions.
You need a complete pipette setup (1 µl to 5 ml) and two 8-channel micro-pipettes. The 8-channel pipettes are used for loading antibodies and for the washing steps.

1- Dissolve your freeze-dried samples (from the Bond Elute filtration) in 600 µl PBS. Vortex to dissolve, leave for 5 min on ice, and then vortex again to make sure all is solved. Centrifuge the samples at 2000 rpm for 5 min to sediment any particles.

2- Mix 270 µl of your samples with 165 µl *P-Ab working solution* in 1.5-ml Eppendorf tubes. Mix 270 µl of each of the 8-oxo-dG standards (0.01, 0.1, 0.5, 1.0, 3.0, and 10 ng/ml) with 165 µl *P-Ab working solution* in 1.5-ml Eppendorf tubes. Vortex gently, spin down the samples for 15 seconds to collect the solution at the bottom of the tubes, then place the samples in a shaker and incubate for 120 min at 37 ˚C while shaking. When the incubation is complete place the samples on ice for 10 min.

3- Directly after step 2, prepare the wells as follows. Wash the wells with 360 µl cold PBS using the 8-channel pipette. Empty the wells by inverting the plate over the sink/, repeat twice. Finally, turn the plate upside down and shake "gently" to remove the last drops. Keep the plate at +4 ˚C until samples are ready (step 2).

4- Transfer 140 µl of the samples/standards (from step 2) in triplicate to the wells according to your labeling design. Seal the plate carefully with the plastic cover. Shake gently at +4 ˚C overnight.

Next day

5- Empty the wells by inverting and shaking it over the sink. All steps can now be performed at room temperature (24 ˚C).

6- Wash the wells three times with *ELISA-washing solution* using the 8-channel pipette as follows: 200 µl – 200 µl – 380 µl. Finally, turn the plate upside down and shake "gently" to remove the last drops.

7- Add 140 µl *S-Ab working solution* to each well using the 8-channel pipette. Cover the plates with the plastic cover and incubate for 2 h at 24 ˚C in the dark.

8- Empty the plate over the sink. Wash three times with *ELISA washing solution* using a 8-channel pipette as follows; 200µl – 200µl – 380 µl. Finish by washing with 200 µl PBS. Empty the plate.

9- Add 140 µl of *Staining solution* using the 8-channel pipette. Shake in the dark for 15-20 min at 24 ˚C. The wells will now turn blue. Within one minute, add 70 µl 1.5 M H_3PO_4 (not included in the kit) using the 8-channel pipette. Shake at room temperature for 3 min (does not have to be in the dark) in a shaker. The samples will now turn yellow. Measure the absorbance at 450 nm within 30 minutes.

10- Based on your standard curve calculate the of 8-oxo-dG concentration of your samples (you can download a program from our homepage).

Example of how to load samples

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2								
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10								
11								
12								