

2015 10

Preparation of solutions and reagents

The Primary antibody stock solution, 8-oxo-dG standard and the Washing solution should be kept at -20 °C for storage all other solutions can be kept at +4 °C. During shipping the kit can be kept at +4 °C.

Solutions needed for the Bond Elut filtration steps

Materials and solutions needed which are included in the kit:

Solvent 1 working solution: The content of the bottle marked *Solvent 1* should be mixed with 500 ml ddH₂O.

Solvent 1A working solution: The content of the bottle marked *Solvent 1A* should be mixed with 10 ml ddH₂O.

Solvent 2 working solution: The content of the bottle marked *Solvent 2* should be mixed with 120 ml ddH₂O and 6 ml methanol.

All these solutions can be stored at +4 °C for two weeks.

Solution for sample preparation

Sample diluent: Ready to use.

Materials and solutions needed for filtration but not supplied

Bond Elut columns (order from Health Biomarkers Sweden)

Vacuum filter holder (order from Health Biomarkers Sweden)

Pipettes from 1 µl to 5 ml

Eppendorf tubes 1.5 and 2 ml

Speed Vac or corresponding (for freeze drying)

Double distilled water (ddH₂O)

Methanol (95 – 99%)

Preparation of solutions for ELISA assay

8-oxo-dG standards in sterile PBS: Upon receipt, aliquot and store the *8-Oxo-dG 100 ng/ml standard* at -20 °C to avoid multiple freeze/thaw cycles. From one of these aliquots 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 standards 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 tube (20 µl) with 1

ml of sterile PBS, transfer the solution to a 10-ml tube and add 4.5 ml of sterile PBS. Store at +4 °C.

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 content of the bottle marked *Washing solution* (22 ml) with 220 ml sterile PBS. Can be stored at +4 °C for two weeks

Chromogen working solution: Add 12 µl H₂O₂ (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 to 15 ml adding 25 µl TMB and 25 µl *Chromogen working solution* (with H₂O₂). Should be used within 1 hour after preparation. (Do not prepare more than you need).

Materials and solutions needed for the ELISA assay but not supplied

Sterile PBS (1000 ml), ddH₂O, H₂O₂ (12 µl, 30 - 37%), 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 microplates.

For additional information contact
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