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In vitro Generation and Quantification of AGEs

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Protocol status: Working

We use this protocol and it's working well.

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Abstract

A seven-step protocol provides how to generate and quantify AGEs *in vitro*.



In vitro Generation and Quantification of AGEs

- 1 To generate Advanced Glycation End Products (AGEs), 10 mL of human serum is incubated with 900 mg of D-glucose (Merck, Melbourne, Australia) at 37 °C in 5% CO₂ atmosphere for 4–6 weeks.
- 2 The AGEs are dialyzed using Slide-A-Lyzer G3 dialysis cassettes (ThermoFisher Scientific, Melbourne, Australia) (10K molecular weight cut-off capacity) that allow de-glucose in the samples.
- 3 Sample (3 mL) is inserted in a lyzer device and stored in PBS buffer with a pH of 7.4 at 4 °C.
- 4 The buffer exchanges are performed every 24 hours.
- 5 Final sample of AGEs is collected after 3-day dialysis.
- 6 Sample of AGEs is stored in a freezer at -20 °C.
- 7 The concentration of AGEs in the human serum samples and control (commercial AGE-BSA) is measured using the AGE Assay Kit (ab238539; Abcam, Cambridge, UK), following the manufacturer's instructions.