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ab219802

Glycated Protein Detection Kit

For the detection of glycated proteins (AGEs) using a simple polyacrylamide gel electrophoresis-based method.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

ab219802 enables the highly sensitive detection of glycated proteins (advanced glycation endproducts, AGEs) using a simple polyacrylamide gel electrophoresis (PAGE)-based method. This kit is compatible with a range of biological samples, including lysates, plasma, sera and tissue homogenates.

Glucose and other metabolites of glycolysis react directly with important cellular components such as DNA, lipids and proteins through a process known as glycation. During glycation, reducing sugar molecules react with the amino groups of amino acids such as those found on lysine, arginine and protein N-termini, ultimately leading to the formation of complex and stable AGEs.

This kit uses Fluorescein-phenylboronate gel electrophoresis (Flu-PAGE) to detect early glycation adducts on proteins by exploiting the reversible covalent interaction between boronic acid and cis-diols that are present in fructosamine-protein adducts in glycated proteins (Pereira Morais et al., 2013). This interaction is further strengthened by the additional charge interaction between boronate and the fructosyllysine amino group (Morais et al., 2010). As the anomeric cis-diols produced by this interaction are absent in N- and O-glycosylation, this method enables the specific identification of glycated proteins over glycosylated and unmodified proteins (Pereira Morais et al., 2013; Kassar et al., 2017). This highly sensitive method detects the earliest stages of glycation, before AGEs are developed, and thus is an ideal tool for identifying reducing sugar modified proteins in complex biological samples such as plasma and brain homogenates.

Pereira Morais, M. P. *et al.* (2013) Analysis of protein glycation using fluorescent phenylboronate gel electrophoresis. *Scientific Reports* **3**, 1437

Morais, M. P. *et al.* Analysis of protein glycation using phenylboronate acrylamide gel electrophoresis. *Proteomics* **10**, 48 (2010).

Kassar. O. *et al.*, Macrophage Migration Inhibitory Factor is subjected to glucose modification and oxidation in Alzheimer's Disease. *Scientific Reports* **7**, DOI: 10.1038/srep42874.

2. Protocol Summary

Determine sample protein concentration



Incubate sample with fluorescein



Analyze sample by SDS PAGE

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website www.abcam.com.

4. Storage and Stability

Store kit at -20°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Fluorescein	1 x 1 mg	-20°C	-20°C
Fluorescein-boronic acid	1 x 1 mg	-20°C	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- 100% methanol
- Protein quantification assay e.g. BCA protein assay kit reducing agent compatible (test tube) (ab207004)
- Buffer at pH 8.0
- 2X SDS PAGE loading buffer (either use 3% w/v SDS, 10% glycerol, 62.5mM Tris-HCL pH 6.8, 0.01% bromophenol blue or Optiblot LDS Sample Buffer (4X) (ab119196) diluted with an equal volume of water). Add 5% β -mercaptoethanol for reducing gels.
- Tris-Glycine SDS polyacrylamide protein gel
- Tris-Glycine SDS running buffer (for example, 25mM Tris-base, 190mM Glycine, 0.1% w/v SDS, pH 8.3)
- SDS PAGE molecular weight markers
- Coomassie Blue or silver stain
- Blue light transilluminator (wavelength range 420–520nm, with amber (530nm) or orange (595nm) filters) or UV transilluminator (wavelength 365nm, with orange (595nm) or green (537nm) filters)

8. Technical Hints

- **This kit contains enough reagent to run 1000 SDS PAGE samples, assuming that the volume of each sample prior to adding loading buffer is 10 μ L.. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample and reagent additions.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 Fluorescein-boronic acid:

Prepare a 10 mg/mL stock solution by adding 100 μ L of 100% methanol to the vial. Resuspend by pipetting up and down. Aliquot and store at -20°C. Avoid multiple freeze-thaw cycles.

9.2 Fluorescein:

Prepare a 10 mg/mL stock solution by adding 100 μ L of 100% methanol to the vial. Resuspend by pipetting up and down. Aliquot and store at -20°C. Avoid multiple freeze-thaw cycles.

10. Assay Procedure

This protocol can be used with a range of biological samples such as lysates, plasma, sera or tissue homogenates.

Reagent volumes in this protocol are provided per SDS PAGE gel sample, assuming that the volume of each sample prior to adding loading buffer is 10 µL. Scale the volumes up if larger sample volumes are required for your gel or if running multiple samples or controls in parallel.

10.1 Buffer preparation:

- 10.1.1 Prepare Fluorescein-boronic acid working solution by adding 1 µL of Fluorescein-boronic acid to 10 µL of 100% methanol. Any Fluorescein-boronic acid working solution left over at the end of the experiment may be stored at -20°C for 6 months. Avoid multiple freeze-thaw cycles.
- 10.1.2 Prepare Fluorescein working solution by adding 1 µL of Fluorescein to 10 µL of 100% methanol. Any Fluorescein working solution left over at the end of the experiment may be stored at -20°C for 6 months. Avoid multiple freeze-thaw cycles.

10.2 Sample preparation:

- 10.2.1 For optimal fluorescein-boronic acid labeling, the sample should be prepared and diluted using a buffer solution at pH 8.0. Avoid detergents such as SDS and Triton™ X-100 as they may interfere with the labeling.
- 10.2.2 Determine the protein concentration of the sample using a protein quantification assay such as BCA protein assay kit reducing agent compatible (test tube) (ab207004).
- 10.2.3 Dilute the sample to the optimal protein loading concentration for SDS-PAGE using a buffer at pH 8. Each SDS PAGE sample or control (test) will require 9 µL of sample at an optimal protein loading concentration.

10.3 Glycated protein labelling and PAGE:

- 10.3.1 Add 1 µL Fluorescein-boronic acid working solution to 9 µL of diluted sample.
- 10.3.2 Optional: If running a control in parallel, prepare the control sample by adding 1 µL Fluorescein working solution to 9 µL of diluted sample.
- 10.3.3 Incubate for 1 hour at room temperature.

10.3.4 Add 10 µL of 2X SDS-PAGE loading buffer.

Δ Note: Fluorescein-boronic acid labeling can be performed under both denaturing and non-denaturing conditions.

10.3.5 Denature and reduce the labeled protein SDS PAGE samples by heating at 85°C for 5 to 10 minutes.

Δ Note: For some samples, better results may be obtained by heating at a lower temperature such as 50°C or by omitting the heating step altogether.

10.3.6 Load equal amounts of protein onto the wells of a Tris-Glycine SDS-PAGE gel, along with a molecular weight marker.

Δ Note: Optimal results are obtained with Tris-Glycine/SDS gels and running buffer at pH 8.3. Bis-Tris, MES, MOPS or other neutral and low pH SDS PAGE gels and running buffer conditions are not recommended. We recommend using molecular weight markers that are easily resolved with blue light or UV light.

10.3.7 Run the gel for 1-2 hours, following the manufacturer's guidelines. Do not stop or interrupt electrophoresis until most of the free fluorescein-boronic acid / fluorescein and the dye front has migrated off the bottom of the gel.

10.3.8 Cut off and discard the bottom section of the gel that contains excess fluorescein-boronic acid and rinse the gel in water.

10.3.9 Visualise the fluorescein-boronic acid labeled glycosylated protein bands by placing the gel on a blue light transilluminator with an amber or orange filter screen. A UV transilluminator with an orange or green filter may also be used. Adjust the contrast and brightness of the gel images to optimize the visibility of the fluorescein boronic acid-labeled glycosylated protein bands.

10.3.10 The gels can be used for further analyses such as anti-AGE or anti-carboxymethyl lysine (CML) Western blotting or total protein staining with Coomassie Blue or silver stain.

11. Typical data

Data provided for **demonstration purposes** only.

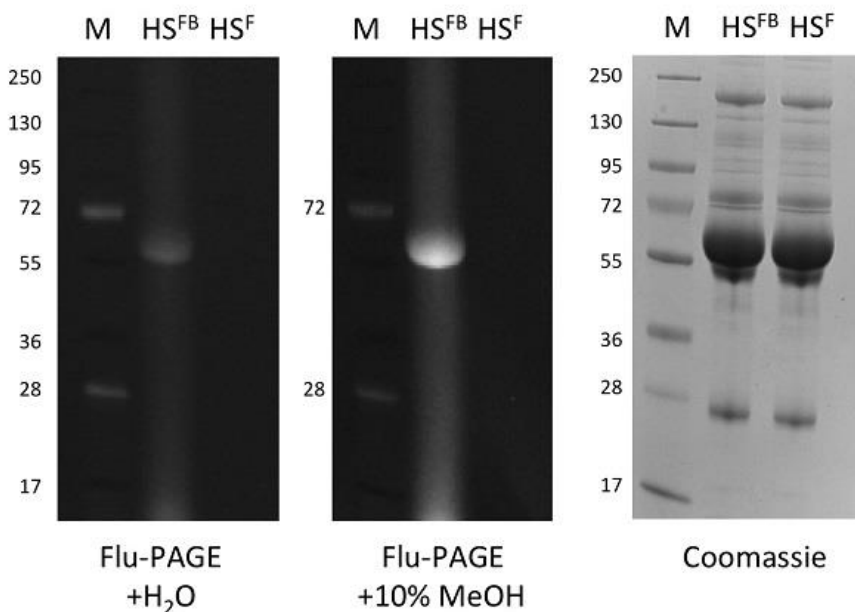


Figure 1. Detection of glycated human serum albumin in normal human serum by Flu-PAGE analysis using Glycated protein detection kit ab219802. A strong band corresponding to glycated Human Serum Albumin is present in fluorescein-boronic acid-treated samples (HS^{FB}) but not in control fluorescein samples (HS^F). Left: Flu-PAGE analysis of normal human serum incubated with a fluorescein-boronic acid working solution made with water. Middle: Flu-PAGE analysis of normal human serum incubated with a fluorescein-boronic acid working solution made with methanol. Right panel: Coomassie Blue staining of the Flu-PAGE gel used to create the image in the centre. Flu-PAGE gels were visualized with a blue light transilluminator using an orange (595nm) filter. Normal human serum was diluted 1:10 in buffer prior to labeling with fluorescein-boronic acid or fluorescein. M – prestained molecular weight markers.

12.Troubleshooting

Problem	Reason	Solution
Low/no signal for protein of interest	Protein of interest is sensitive to the presence of methanol.	Prepare the fluorescein-boronic acid working solution by diluting the fluorescein-boronic acid stock solution in double-distilled water instead of 100% methanol. Note that, typically, staining is less intense when the working solution is prepared using water instead of 100% methanol.
	Protein of interest is sensitive to proteolysis.	Try incubating the sample with fluorescein-boronic acid overnight at 4°C instead of for 1 hour at room temperature.
	Type of starting material.	Some tissue homogenates require longer staining. Try incubating the sample with fluorescein-boronic acid overnight at 4°C instead of for 1 hour at room temperature.
	Protein of interest has low molecular weight.	The intensity of free fluorescein-boronic acid at the bottom of the gel may interfere with the detection of low molecular weight glycosylated proteins. Remove excess fluorescein-boronic acid after incubation by passing the sample over a desalting column prior to electrophoresis.
High background signal	Excess fluorescein-boronic acid during SDS-PAGE	Remove excess fluorescein-boronic acid after incubation by passing the sample over a desalting column prior to electrophoresis.

13. Notes

Technical Support

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