Amplite™ Rapid Colorimetric Protein Aldehyde Content Quantitation Kit

Catalog number: 5536
Unit size: 2 Tests

Component	Storage	Amount
Component A: AldeView™ 488	Freeze (< -15 °C), Minimize light exposure	2 vials
Component B: Reaction Buffer	Refrigerated (2-8 °C)	1 vial (30 µL)
Component C: Spin Column	Room temperature (10-25 °C)	2 columns

OVERVIEW

Site-selective modification of proteins is of major interest in chemical biology. Most of the traditional methods used for protein modifications are based on reactions of amine or thiol groups within a protein. However, many methods have been developed to incorporate aldehyde functional groups into proteins. Rapid and accurate measurement of aldehyde content is an important tool for biological and chemical research. Amplite™ Rapid Colorimetric Protein Aldehyde Content Quantitation Kit provides an accurate method to quantify aldehyde groups using our proprietary AldeView™ 488 sensor, which has the maximum absorbance at ~495nm. AldeView™ 488 reacts with the aldehyde-modified protein samples. The resulted product is run through a single spin column to remove the excess AldeView™ 488 sensor. The absorbance spectra of purified product are measured, and the aldehyde to protein ratio can be determined via the absorbance ratio of 495 nm/280 nm. Amplite™ Rapid Colorimetric Protein Aldehyde Content Quantitation Kit can be performed in a traditional cuvette, NanoDrop™ Spectrophotometer or a convenient 96-well absorbance plate reader with a UV-transparent plate.

AT A GLANCE

Protocol summary

1. Prepare 100 µL of protein sample
2. Add 10 µL of Reaction Buffer (Component B) to protein sample
3. Mix protein sample and AldeView™ 488 (Component A)
4. Rotate at room temperature for 60 minutes
5. Purify protein with a spin column
6. Monitor the absorbance of the elution solution and measure aldehyde contents

Important

When stored properly, the kit components should be stable for six months. Do not freeze Spin Column (Component C). Warm up all the components before the experiments. 50 to 100 µg of protein sample is needed for determining the amount of aldehyde content.

KEY PARAMETERS

NanoDrop

Absorbance	280 nm and 495 nm

Spectrophotometer

Absorbance	250-750 nm
Recommended plate	Cuvette

Absorbance microplate reader

Absorbance	280 nm and 495 nm
Recommended plate	Clear bottom

SAMPLE EXPERIMENTAL PROTOCOL

Preparation of Sample Solution

1. Adjust the volume of 50 to 100 µg of protein sample to 100 µL with PBS.

2. Add 10 µL Reaction Buffer (Component B) to the protein sample and mix well. Total protein sample volume is 110 µL.

Run Aldehyde Assay

1. Add the protein sample to one vial of AldeView™ 488 (Component A).

2. Mix well by pipetting for a few times or vertexing the vial for a few seconds.

3. Keep the reaction mixture at room temperature and rotate or shake for 60 minutes.

Prepare Spin Column for Sample Purification

1. Invert the Spin Column (Component C) several times to re-suspend the settled gel and remove any air bubbles.

2. Snap off the tip and place the column in a wash tube (2 mL, not provided). Remove the cap to allow the excess packing buffer to drain by gravity to the top of the gel bed. If column does not begin to flow, push the cap back onto column and remove again to start the flow. Discard buffer, and then place the column back into the wash tube. Alternatively, centrifuge immediately if the column is placed into a 12 × 75 mm test tube (not provided).

3. Centrifuge for 1 minute in a swinging bucket centrifuge at 1,000 × g to remove the packing buffer. Discard the buffer.

4. Apply 1 mL PBS to the column, let the buffer drain out by gravity, or centrifuge the column for 1 minute to remove the buffer. Discard the buffer from the Washing Tube. Repeat this process for 3 – 4 times.

5. Centrifuge in a swinging bucket centrifuge rotor at 1000 × g for 2 minutes to remove the reaction buffer. Discard the buffer.

   Note Spin Column (Component C) can fit into 2 mL microcentrifuge tubes or 12×75 mm test tubes for sample collection during centrifugation. Use the 2 mL microtubes provided with the columns for the initial column equilibration step. Swinging bucket centrifuges capable of generating a minimum force of 1,000 × g is suitable for use with the Bio-Spin column. The gravitational force created at a particular revolution speed is a function of the radius of the microcentrifuge rotor. Consult the swinging bucket centrifuge instruction manual for the information about conversion from revolutions per minute (RPM) to centrifugal or g-force. Alternatively, use the equation to calculate the speed in RPM required to reach the gravitational force of 1,000 × g. RCF (g) = $(1.12 \times 10^{-5}) \times (\text{RPM})^2 \times r$

RCF = the relative centrifugal force, RPM = the speed of the rotor, r = the radius in centimeters measured from the center of the rotor to the middle of the Bio-Spin column.

Purify Reaction Product

1. Place the column in a clean collection tube (1.5 mL, not provided). Carefully load the sample (110 µL) directly to the center of the column.

2. After loading the sample, add 10 µL PBS to the top and centrifuge the column for 5 minutes at 1,000 × g, and collect the solution into the collection tube.

Measure Absorbance

1. Dilute the reaction product 5-folds with PBS depending on the size of the cuvette used and the absorbance reading.

   Note The dilution factor does not affect the final aldehyde quantitation result.

2. Measure the absorption spectrum over the range from 250 nm to 750 nm , or only read the absorbance (OD) at 280 nm and 495 nm.

3. Calculate the aldehyde content on protein:

Constants needed:
Protein extinction coefficient at 280 nm ($\varepsilon_{(protein)}$)
AldeView™ 488 extinction coefficient at maximum absorption (495 ± 3 nm): $\varepsilon_{(sensor)}$ = 75,000 M^{-1} cm^{-1}
Correction Factor of AldeView™ 488 at 280 nm: CF_{280} = 0.117
Aldehyde Calculation:
(Moles of Aldehyde/Moles of protein) = $(A_{495} / \varepsilon_{(sensor)}) / [(A_{280} – CF_{280} \times A_{495})/ \varepsilon_{(protein)}]$

EXAMPLE DATA ANALYSIS AND FIGURES

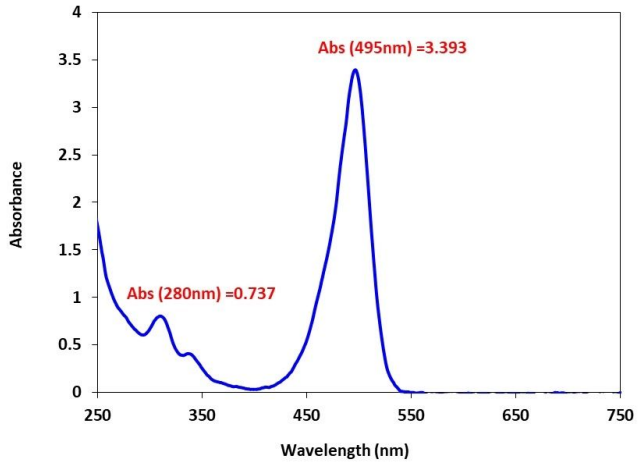

Figure 1. Aldehyde quantitation of BSA-Acrolein Conjugate with Amplite™ Rapid Colorimetric Protein Aldehyde Content Quantitation Kit. Absorbance spctrum was measured with NanoDrop Spectrometer.
Aldehyde/BSA = ((A495/ 75000) / [(A280 – 0.117 × A495)/ 43824] = 5.8

DISCLAIMER

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