



## Rapid Colorimetric Surface Protein Test: Determination of the Limit of Detection for AllerSnap® Devices

### Application

AllerSnap® tests are quick and easy ways to detect allergenic and non-allergenic protein residues left on a surface after cleaning. Simply swab a surface, release the reagent and incubate. If food residue containing protein is present, the reagent will turn from green to purple. The color change provides a semi-quantitative measure for such residues. The more protein present, the quicker the color change to purple and the darker the color. AllerSnap quickly measures the contamination of a surface, allowing immediate corrective action to be taken, if necessary.

### Principle

The test is based on the principle of the Biuret reaction where, under alkaline conditions, copper (II) ions form a complex with the peptide bonds of proteins and are reduced to copper (I) ions. Bicinchoninic acid (BCA), under alkaline conditions, is a highly sensitive, stable and specific reagent for copper (I) ions, forming a purple complex when bound to copper (I) ions. The chromogen formed can be assessed visually with the AllerSnap device.



AllerSnap tests detect peptide bonds, which are present in all proteins, so it does not discriminate between the types or sizes of proteins detected.

### Performance

The reaction is time-dependent, i.e., the color develops with time. Therefore, it is important to record the color change up to 30 minutes when incubated at 37 °C. A positive result for protein residue can be noted as soon as any color change is observed, but any color change after 30 minutes should be disregarded.

The precision of the reaction is also temperature-dependent: it is important to allow the devices to equilibrate to room temperature (15 to 25 °C) if they have been stored at a different temperature. Following such equilibration, the devices can be then incubated according to instructions.

### False Positives

This test can detect other substances capable of reducing copper (II) ions to copper (I) ions, such as reducing sugars (glucose) and uric acid. Other strong reducing materials, such as ascorbic acid (present in some fruit juices) or tannin (present in tea), may also give a positive result with AllerSnap devices.

### Sensitivity

AllerSnap devices detect 3 µg of protein when samples are incubated for 30 minutes at 37 °C.

Table 1 shows the color change associated with various dilutions of protein-derived allergens (µg/10 µL, where 1 µg/mL equals 1 ppm).



**Table 1. How to Interpret the Color Indicator for AllerSnap Devices.**

Color change	Level	Result	Detection
Green	1	Pass	<3 µg
Grey-green	2	Caution	3 – 5 µg
Grey	3	Fail	6 – 10 µg
Light purple	4	Fail	11 – 25 µg
Dark purple	5	Fail	≥26 µg

### Purpose

This study was conducted to determine the limits and linearity of response for protein detection by AllerSnap devices.

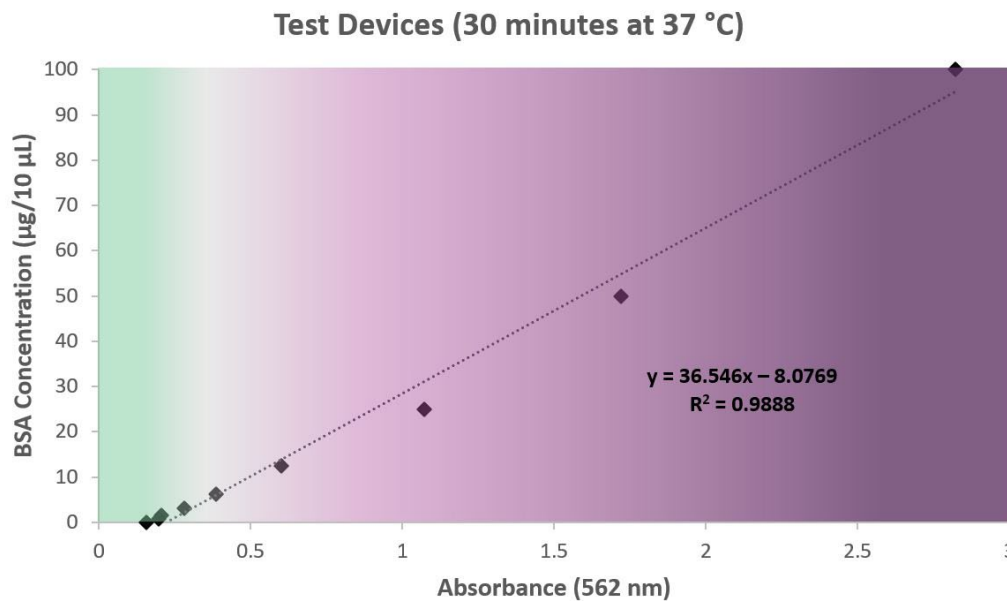
### Evaluation

Bovine Serum Albumin (BSA) protein was tested during 30-minute incubations at 37 °C.

### Materials and Methods

- Incubators specific for such devices were set at 37 °C. (i.e., Hygiena’s Dry Block Incubator, Product No. INCUBATOR)
- In-date AllerSnap devices were used throughout the testing.
- In addition to color observations, the absorbance of the BCA-Cu<sup>+</sup> complex was recorded at 562 nm.
- Concurrent determinations were performed in glass test tubes using the same reagents and volumes as the AllerSnap devices.

### Results



**Figure 1. Standard curve for BSA in AllerSnap devices incubated for 30 minutes at 37 °C shows a 98.88% correlation.**

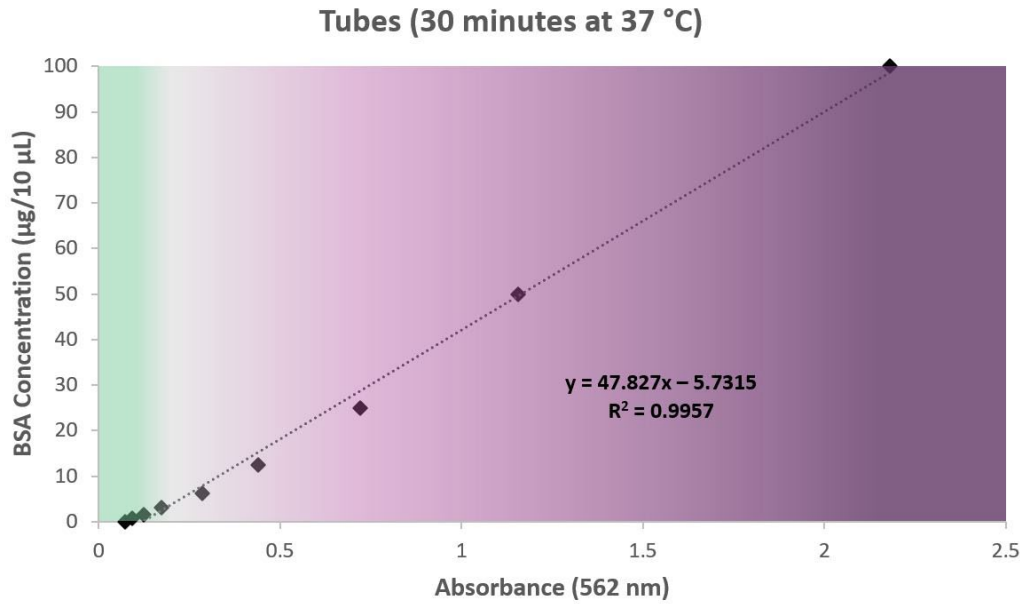


Figure 2. Standard curve for BSA in glass test tubes incubated for 30 minutes at 37 °C shows a 99.57% correlation.

### Observations

AllerSnap devices can be used to detect the presences of allergenic and non-allergenic proteins at a wide range of concentrations. These recyclable devices will save time and resources compared to testing in glass tubes.

- The detection limit is 3 µg of BSA protein.
- Results are linear across a wide range of protein concentrations.
- There is good correlation between protein determination in AllerSnap devices and in test tubes.