

## Rapid Colorimetric Surface Protein Test: Determination of the Limit of Detection for PRO-Clean

### Application

PRO-Clean is a quick and easy way to monitor the cleanliness of food equipment surfaces to help ensure food safety and product quality. PRO-Clean detects protein residues left on a surface after cleaning. Simply swab a surface, release the reagent and if food residue containing protein is present the reagent will turn purple. The color change provides a semi-quantitative measure of the surface cleanliness. The more protein present, the quicker the color change to purple and the darker the color. PRO-Clean quickly validates the hygiene of a surface, allowing immediate corrective action to be taken if necessary.

### Principles

The test is based on the principle of the Biuret reaction where under alkaline conditions the copper ions ( $\text{Cu}^{2+}$ ) form a complex with the peptide bonds of proteins and becomes reduced to copper  $\text{Cu}^+$ . Bicinchoninic acid (BCA) under alkaline conditions is a highly sensitive, stable and specific reagent for  $\text{Cu}^+$  forming a purple complex. The chromogen formed can be assessed visually with the PRO-Clean device.



### Test Performance

The reaction is time and temperature dependant, i.e. the color develops with time and the speed of color development is slower or faster depending on the temperature. We recommend that results be read within 10 minutes of activation at room temperature, and then discarded. The reaction takes place in minutes depending on the level of contamination. The test chemistry will turn to purple over a long period of time (4-5 hours) even with the absence of protein. Users may interpret the level of contamination on a surface based on the speed and intensity of the color change.

Since the reaction is temperature-dependent, it is important to allow the devices to equilibrate to ambient room temperature (15 - 25°C) if they have been stored at refrigerated temperatures.

The test also detects other substances capable of reducing the copper ( $\text{Cu}^{++}$  to  $\text{Cu}^+$ ) 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 and coffee) may also give a positive result with PRO-Clean.

### Sensitivity

A dilution series of a known purified protein solution (Bovine Serum Albumen, BSA) was prepared covering a range of 0 – 100 µg (micrograms). A 10 µl aliquot was added to each of three replicate PRO-Clean devices that were activated. The color development was assessed visually at 1, 5 and 10 minutes at room temperature. The color changes were interpreted and recorded using the color chart below and the results are shown in Table 1.

**Visual color interpretation of PRO-Clean results**


Color	PASS/FAIL	Value
Light Green	PASS	1
Grey/Light Purple	FAIL	2
Light Purple	FAIL	3
Dark Purple	FAIL	4

**Table 1 PRO-Clean Color Change Over 10 minutes at Room Temperature ( 20 – 23C)**

Protein (BSA, µg)	1 minute	1 min Value	5 minutes	5 min Value	10 minutes	10 min Value
100µg	FAIL	2	FAIL	4	FAIL	4
90µg	FAIL	2	FAIL	4	FAIL	4
80µg	FAIL	2	FAIL	3	FAIL	4
70µg	PASS	1	FAIL	3	FAIL	4
60µg	PASS	1	FAIL	2	FAIL	3
50µg	PASS	1	FAIL	2	FAIL	3
40µg	PASS	1	PASS	1	FAIL	3
30µg	PASS	1	PASS	1	FAIL	3
20µg	PASS	1	PASS	1	FAIL	2
10µg	PASS	1	PASS	1	PASS	1
5µg	PASS	1	PASS	1	PASS	1
1µg	PASS	1	PASS	1	PASS	1

**The limit of detection of PRO-Clean is**

- 80 µg protein after 1 minute at room temperature
- 50 µg protein after 5 minutes at room temperature
- 20 µg protein after 10 minutes at room temperature

## Detection of Protein from Foods

A range of foods were selected for analysis to determine the limit of detection of PRO-Clean. A 10% suspension was prepared from solid foods by homogenizing 10g with 90ml sterile water. This was coarsely filtered and diluted further to make suspensions containing 1, 0.1 and 0.01 % for each food. A 10 µl aliquot of each diluted food suspension was added to each of three replicate PRO-Clean devices that were activated. The color development was assessed visually at 1, 5 and 10 minutes at room temperature. The results are shown in Table 2 below. (Neat= 10% suspension, and 1/ 10 = 1% suspension of food residue).

**Table2 PRO-Clean Detection of Food at Room Temperature**

Product	Dilution	Detected	Time to Results	Comments
Ground Beef	Neat	YES	10	Level 4
Beef Steak	Neat	YES	10	Level 4
Processed Beef	1/10	YES	5	Level 3
Processed Ham	Neat	YES	10	Level 2
Raw Chicken	1/10	YES	5	Level 4
Cooked Chicken	1/10	YES	5	Level 3
Raw Eggs	Neat	YES	10	Level 4
Shrimp	1/10	YES	10	Level 3
Fish	1/10	YES	10	Level 4
Pasteurized Milk	1/10	YES	10	Level 4
UHT Milk	1/10	YES	10	Level 4
Cream	1/10	NO	-	<i>Inhibition</i>
Cheddar Cheese	1/10	YES	5	Level 4
Cottage Cheese	1/10	YES	10	Level 4
Yogurt	1/10	YES	10	Level 3
Margarine	Neat	NO	-	<i>Inhibition</i>
Cola	1/10	YES	10	Level 3
Orange Juice	1/10	YES	5	Level 4
Cranberry Juice	1/10	YES	5	Level 4
Pineapple	1/10	YES	5	Level 4
Banana	1/10	YES	5	Level 4
Chocolate	1/10	YES	5	Level 4
Walnut	1/10	YES	5	Level 4
Peanut	1/10	YES	10	Level 4
Soybean	1/10	YES	10	Level 3

## Conclusions

PRO-Clean was able to detect a wide variety of food types giving strong color reaction in 5 – 10 minutes. Foods with a high fat content and low protein content could not be detected. The limit of detection (Level 2 after 10 minutes) for most foods tested occurred at the 1/ 10 – 1/100 dilution level i.e. 1.0 to 0.1% of product.