PRO-Clean® Rapid Colorimetric Surface Protein Test Sensitivity and Multi-Matrix Performance

PRO-Clean Cleaning Verification Method

The Hygiena PRO-Clean surface protein test is a simple method to verify and monitor the cleanliness of food equipment surfaces as a component of food safety and HACCP processes. PRO-Clean detects protein residues with a simple swab and reagent release step. Surface residual proteins will react to form a semi-quantitative color change with higher protein levels resulting in more rapid, darker purple color development. PRO-Clean's speed and accuracy allows for fast and reliable cleaning verification as well as immediate corrective action when indicated.

PRO-Clean Test Principle

PRO-Clean utilizes the Biuret reaction where under alkaline conditions copper ions (Cu^{2+}) form a complex with protein peptide bonds[1] resulting in reduced copper Cu^{+}. The Cu^{+} specific bicinchoninic acid (BCA) reagent then forms a purple chromogen complex which is read visually.

$$\text{Protein} + Cu^{2+} \xrightarrow{OH^-} \text{Protein-}Cu^{+} \xrightarrow{+BCA} \text{Purple Complex}$$

The Biuret reaction is time, temperature, and concentration-dependent. As such, PRO-Clean devices should be used at room temperature (15 - 25 °C) and read within 10 minutes[2]. High protein concentration and increased incubation (temperature or time) will increase the speed and intensity of the reaction.

PRO-Clean Test Sensitivity (Limit of Detection)

PRO-Clean sensitivity was determined by testing a series of diluted bovine serum albumin (BSA) samples with a range of 0 - 100 µg. Three (10 µl) replicates of each sample were tested at room temperature. Visual assessments were made at 1, 5 and 10 minutes referencing the PRO-Clean color chart (Figure 1). Test results (Table 1) confirm a 20 µg protein limit of detection (LOD) at the recommended 10-minute, room temperature read time.

Figure 1. PRO-Clean Color Chart for Results Interpretation

Color	Pass/Fail	Value
Light Green	Pass	1
Grey/Light Purple	Fail	2
Light Purple	Fail	3
Dark Purple	Fail	4
✓	X	X	X

Table 1. PRO-Clean LOD Data (diluted BSA tested at 20 - 23 ˚C)

BSA Protein Concentration	1 Minute Read Time	Detection Level (Value at 1 min)	5 Minute Read Time	Detection Level (Value at 5 mins)	10 Minute Read Time	Detection Level (Value at 10 mins)
100 µg	Fail	2	Fail	4	Fail	4
90 µg	Fail	2	Fail	4	Fail	4
80 µg	Fail	2	Fail	3	Fail	4
70 µg	Pass	1	Fail	3	Fail	4
60 µg	Pass	1	Fail	2	Fail	4
50 µg	Pass	1	Fail	2	Fail	3
40 µg	Pass	1	Pass	1	Fail	3
30 µg	Pass	1	Pass	1	Fail	2
20 µg	Pass	1	Pass	1	Fail	2
10 µg	Pass	1	Pass	1	Pass	1
5 µg	Pass	1	Pass	1	Pass	1
1 µg	Pass	1	Pass	1	Pass	1

PRO-Clean Test Multi-Matrix Performance

The PRO-Clean method was also challenged by using a variety of food matrices to simulate real-world testing conditions. Each locally sourced food type was weighed, placed into a sterile container, and homogenized via physical means or stomaching. For solid foods, 10 µg was combined with 100 mL of sterile water to create a 10% solution. The solution was then coarsely filtered to remove food debris, resulting in a liquid homogenate (neat sample). The homogenate was also further diluted to a 1% sample concentration (1:10 sample). Three (10 µl) replicates of each diluted sample were tested at room temperature according to PRO-Clean instructions for use. Visual assessments were made at 1, 5 and 10 minutes referencing the PRO-Clean color chart (Figure 1). Test results are summarized in Table 2 below.

Table 2. PRO-Clean Protein Residue Test Results by Matrix

Product[3]	Homogenate Concentration (Dilution)	Detected	Read Time (Minutes)	Detection Level (Value)
Ground beef	10% (neat)	Yes	10	4
Beef steak	10% (neat)	Yes	10	4
Processed beef	1.0% (1:10)	Yes	5	3
Processed ham	10% (neat)	Yes	10	2
Raw chicken	1.0% (1:10)	Yes	5	4
Cooked chicken	1.0% (1:10)	Yes	5	3
Raw eggs	10% (neat)	Yes	10	4
Shrimp	1.0% (1:10)	Yes	10	3
Fish	1.0% (1:10)	Yes	10	4
Pasteurized milk	1.0% (1:10)	Yes	10	4
UHT milk	1.0% (1:10)	Yes	10	4
Cheddar cheese	1.0% (1:10)	Yes	5	4
Cottage cheese	1.0% (1:10)	Yes	10	4
Yogurt	1.0% (1:10)	Yes	10	3
Cola	1.0% (1:10)	Yes	10	3
Orange juice	1.0% (1:10)	Yes	5	4
Cranberry juice	1.0% (1:10)	Yes	5	4
Pineapple	1.0% (1:10)	Yes	5	4
Banana	1.0% (1:10)	Yes	5	4
Chocolate	1.0% (1:10)	Yes	5	4
Walnut	1.0% (1:10)	Yes	5	4
Peanut	1.0% (1:10)	Yes	10	4
Soybean	1.0% (1:10)	Yes	10	3

Conclusion

In addition to the validated 20 µg protein (BSA) limit of detection, PRO-Clean demonstrates accurate detection of residual protein (1 - 10%) across a wide range of food matrices. This data serves to validate its cleaning verification utility for a broad range of food processors and environments.

As an industry leading tool for rapid cleaning verification, PRO-Clean offers a simple, sensitive and reliable method for detecting residual food proteins, thereby establishing its value in effective HACCP program management.

---

[1] The Biuret method also detects other copper reducing substances, including glucose, uric acid, ascorbic acid, and tannins.

[2] Always read test results within 10 minutes of swabbing as the Biuret test chemistry will appear purple over an extended period (4 - 5 hours) regardless of protein levels.

[3] Cream and margarine samples inhibited the Biuret reaction.