ELISA procedure for detecting binding media in paint

Place 10 to 500 μg of each sample to be analyzed into 2-mL micro-centrifuge tubes. Add 20 μL of elution buffer to each sample. Elution Buffer: 5 mL of 1M tris (hydroxymethyl)aminomethane hydrochloride (tris-HCl), 1 mL of 0.5 M ethylenediaminetetraacetic acid (EDTA), 180 g urea, 25 mL of 20% sodium dodecyl sulfate (10 g in 50 mL deionized water), and deionized H2O final volume 500 mL. EB pH adjusted to 7.4 using NaOH. The solution may be stored at room temperature.

Prepare positive controls: Depending on the assay, add 10 to 50 μg each of egg white, rabbit skin glue, animal glue (typically bovine), cow's milk (casein), or gum Arabic into separate micro-centrifuge tubes. Add 20 μL elution buffer to each positive control and also to a sterile "blank" tube, containing no antigen. Allow samples, standards, and blank to elute by standing for 2–3 days at room temperature (minimum 12 hours).

Next, add 80 μL of 100 mM sodium bicarbonate to each tube. 100 mM sodium bicarbonate solution: 0.42 g of NaHCO3 brought to a total of 50 mL with deionized H2O; the solution may be stored at room temperature. Mix well and let sit for 10 minutes; this allows any particulate matter settles to bottom of the tube. Prepare the ELISA plates for the samples by adding 40 μL of bicarbonate buffer to each of the wells in two separate 96-well polystyrene

ELISA plates. ELISA plates are typically labeled with eight rows (A-H) and 12 columns (1–12).

Multiple dilutions for each sample are used to verify the results of the assay. Dilutions can be obtained by direct pipette transfer between rows as well or by adding 40 µL of eluent from the first sample to A1, 20 µL to B1, 10 µL to C1, and 5 µL to D1. A minimum of 4 dilutions are recommended. Repeat this procedure for each subsequent sample by adding eluent to the successive columns of the plates, reserving columns 11 and 12 for blanks and positive controls. Dilutions can be obtained by direct pipette transfer between rows as well.

Next, add 40 µL of blank eluent solution to all the wells in column 12. After the blanks, add the positive controls to column 11. Add bicarbonate buffer to each of the wells so that the final volume is 80 µL.

Cover the plates with ParafilmTM and incubate at 4 °C for 24 hours. After incubation, allow the plates to come to room temperature and empty the contents of each well with a multi-channel pipette (#L300S, Rainin Instrument, LLC, Oakland, CA USA). Be cautious at this step, as it is possible to cross-contaminate nearby wells. If necessary, dry the pipette tips on clean absorbent paper between rows.

Rinse the wells by adding 300 µL of phosphate buffered saline (PBS) to

each well and allow to stand for 2 minutes. All PBS solutions used in this procedure were diluted 1:10 with deionized water from standard commercial 10× concentrate. PBS solutions may be stored at room temperature. Empty the wells into a waste receptacle by inverting the plate with a brisk shake. Repeat this rinsing operation two times. It is important to rinse thoroughly, so that all unbound sample material, including particulate residue, is removed. Tap the plate on a paper towel between rinsing to remove all of the PBS from of the wells. Add 300 µL Sea BlockTM Buffer diluted 1:10 v/v in PBS to all the wells. Allow the trays to stand for 60 minutes at room temperature. Empty the wells into an appropriate receptacle with a flick of the wrist, and pat the inverted plate dry on a paper towel.

Next, add 80 µL of the appropriate diluted primary antibody to each of the wells in the rows described below (Table 1). Dilutions of all the antibodies are prepared using Sea BlockTM Buffer solution mentioned above. Allow the antibodies to bind for 2 hours at room temperature.

Empty the plates with a flick of the wrist, and rinse all wells 3 times with 300 µL of PBS as above. Next, add 80 µL of diluted secondary antibody to each row of wells as follows: Allow the antibodies to bind for 2 hours at room temperature.

Empty the plates and rinse all wells 3 times as before, then add 80 µL of p-nitrophenyl phosphate pNPP solution. Wait until the controls are fully developed while making sure that the blanks remain clear (up to one hour). If desired, stop the reaction by adding 80 µL 0.75 M NaOH to each of the wells. Measure the absorbance at 405 nm of the solution in each well using a spectrophotometer or automated plate reader such as Finstruments model #341 96-well microplate spectrophotometer (MTX Lab Systems, Inc., Virginia USA). In the case of strong responses, the results can be read qualitatively by eye.

Primary Antibody Dilution Used	Secondary Antibody Dilution Used
Egg Ovalbumin #AB1225 800 (5 µL > 4 mL)	Rabbit IgG #AP132A 500 (30 µL > 15 mL)
Collagen #AB6577 200 (10 µL > 2 mL)	Rabbit IgG #AP132A 500 (30 µL > 15 mL)
Collagen #AB198111 400 (5 µL > 2 mL)	Goat IgG #AB6742 400 (5 µL > 2 mL)
Fish Collagen #T89171R 100 (20 µL > 2 mL)	Rabbit IgG #AP132A 500 (30 µL > 15 mL)
Casein, #RCAS-10A 800 (5 µL > 4 mL)	Rabbit IgG #AP132A 500 (30 µL > 15 mL)
Plant gum #JIM 13 50 (40 µL > 2 mL)	Rat IgG AB6846 400 (5 µL > 2 mL)