

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 100 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: Add 10 to 500 μg each of egg yolk, egg white, rabbit skin glue, animal glue (typically bovine), cow's milk (casein), fish glue, gum Arabic, and gum tragacanth into separate micro-centrifuge tubes.² Add 100 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.

Next, add 200 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 settle to bottom of the tube. Prepare the ELISA plates for the samples by adding 65 μL of bicarbonate buffer to each of the wells in two separate 96-well polystyrene

ELISA plates. 4 ELISA plates are typically labeled with eight rows (A-H) and 12 columns (1–12). Plate 1 should be labeled as follows: rows A and B – rabbit skin glue; rows C and D – animal glue; rows E and F – fish glue; rows G and H – casein. Plate 2 should be labeled: rows A and B – egg white; rows C and D – egg yolk; rows E and F – plant gum (general); rows G and H – gum tragacanth. Duplication is recommended to verify the results of the assay.

Add 15 μ L of eluent from the first sample to each of the wells in column 1 for both ELISA plates 1 and 2. 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.

Next, add 15 μ L of blank eluent solution to all the wells in column 11 for both ELISA plates 1 and 2. After the blanks, add the positive controls as follows: in plate 1, add 15 μ L eluent from the rabbit skin control glue to wells 12A and 12B, 15 μ L animal glue eluent to wells 12C and 12D, 15 μ L fish glue eluent to wells 12E and 12F, and 15 μ L casein eluent to wells 12G and 12H. In plate 2, add 15 μ L of eluent from the egg white control to wells 12A and 12B, 15 μ L egg yolk eluent to 12C and 12D, 15 μ L gum Arabic eluent to 12E and 12F, and 15 μ L gum tragacanth eluent to 12G and 12H.

Cover the plates with Parafilm™ 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 μ L 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 \times 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 μ L Sea BlockTM Buffer diluted 1:10 v/v in PBS to all the wells except the undiluted Sea BlockTM Buffer is added to the wells for egg yolk and gum tragacanth (plate 2 rows C, D, G, H). 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 μ L 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. In plate 1, add #AB19811 (collagen) to rows A and B; add #AB6577 (collagen) to rows C and D; add #T89171R (fish collagen) to rows E and F; and add

#RCAS-10A (casein) to rows G and H. In plate 2, add #AB1225 (ovalbumin) to rows A and B; add #SC-46681 (phosvitin) to rows C and D; add #JIM13 (general plant gum) to rows E and F; and add #MAC265 (gum tragacanth) to rows G and H. 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 μ L of PBS as above. Next, add 80 μ L of diluted secondary antibody to each row of wells as follows: In plate 1, add #AB6742 (goat IgG) to rows C and D; add #AP132A (rabbit IgG) to all other rows; In plate 2, add #AP132A (rabbit IgG) to rows A and B; add #AP124A (mouse IgG) to rows C and D; add antibody KPL#05-16-03 (rat IgM) to rows E and F; and add #AB6846 (rat IgG) to rows G and H. 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 μ L 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). Quench the reaction by adding 80 μ L 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
Collagen #AB6577 200 (10 μ L > 2 mL)	Rabbit IgG #AP132A 500 (30 μ L > 15 mL)
Collagen #AB198111 400 (5 μ L > 2 mL)	Goat IgG #AB6742 400 (5 μ L > 2 mL)
Fish Collagen #T89171R 100 (20 μ L > 2 mL)	Rabbit IgG #AP132A 500 (30 μ L > 15 mL)
Casein, #RCAS-10A 800 (5 μ L > 4 mL)	Rabbit IgG #AP132A 500 (30 μ L > 15 mL)
Ovalbumin #AB1225 800 (5 μ L > 4 mL)	Rabbit IgG #AP132A 500 (30 μ L > 15 mL)
Phosvitin #SC-46681 66 (30 μ L > 2 mL)	Mouse IgG #AP124A 400 (5 μ L > 2 mL)
Plant gum #MAC207 50 (40 μ L > 2 mL)	Rat IgM KPL#05-16-03 100 (20 μ L > 2 mL)
Gum Tragacanth #MAC265 50 (40 μ L > 2 mL)	Rat IgG #AB6846 400 (5 μ L > 2 mL)