



Environmental Analysis Program

Pomona College
Biogeochemistry Laboratory

SOP-L307

ELISA: Bisphenol A, Bisphenol B, and Pesticides in Water

Standard Operating Procedure

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1. Purpose

This SOP describes the use of competitive enzyme-linked immunosorbent assay (ELISA) kits to screen water samples for bisphenol A (BPA), bisphenol B (BPB), and selected pesticides (atrazine, glyphosate, and 2,4-D). ELISA provides a rapid, cost-effective immunochemical screening method for detecting trace-level organic contaminants in environmental water samples without the need for extensive sample preparation or chromatographic instrumentation (Aga, 2013; Goda et al., 2000; Voller et al., 1978). Results exceeding the kit detection threshold should be considered presumptive and confirmed by an instrumental method (e.g., LC-MS/MS or GC-MS) when data will be used for regulatory reporting (US Environmental Protection Agency, 1996).

2. Scope

- **Applies to:** Screening of surface water, groundwater, treated effluent, and drinking water source water for BPA, BPB, atrazine, glyphosate, and 2,4-D using commercially available competitive ELISA plate kits (96-well microplate format)
- **Does not apply to:** Confirmatory quantitative analysis by LC-MS/MS or GC-MS (see [SOP-L309](#)); soil or sediment extracts (matrix effects require separate validation); clinical or food-safety testing
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
PI / Lab Manager	Select analytes and approve kit vendors; review QC data
Trained Personnel	Run ELISA assays, record results, flag exceedances
New Students	Complete training before independent use

4. Definitions

- **Absorbance:** The logarithm of the ratio of incident to transmitted light through a colored solution, measured at the wavelength of maximum absorption; the raw output of spectrophotometric ELISA readers.

- **Bisphenol A (BPA):** An industrial chemical used in polycarbonate plastics and epoxy resins; an endocrine-disrupting compound detected in environmental water samples.
- **Competitive ELISA:** An enzyme-linked immunosorbent assay format in which analyte in the sample and a fixed amount of enzyme-labeled analyte compete for binding to a limited number of antibody sites; signal decreases as analyte concentration increases (inverse response).
- **Cross-reactivity:** The degree to which an antibody recognizes structurally similar compounds other than the target analyte; expressed as a percentage and listed in the kit insert; high cross-reactivity with related compounds (e.g., BPB with a BPA kit) can produce false positives.
- **ELISA (Enzyme-Linked Immunosorbent Assay):** A plate-based immunoassay that uses antibody-antigen binding and an enzyme-substrate color reaction to detect and quantify target analytes.
- **Matrix effect:** Interference from sample components (suspended solids, humic acids, pH, salinity) that alter antibody-antigen binding or enzyme activity; minimized by sample dilution, solid-phase extraction, or pH adjustment.
- **Standard curve:** A set of calibration solutions of known analyte concentration used to convert absorbance readings to concentrations; ELISA curves are typically sigmoidal (4-PL fit).

5. Safety and Hazards

Hazards

- **Chemical:** ELISA kits contain dilute acids (stop solution, typically 1 N HCl or H₂SO₄), enzyme conjugates, and substrate solutions (typically 3,3',5,5'-tetramethylbenzidine, TMB). Stop solution is corrosive at working concentration. Review the Safety Data Sheet (SDS) provided with each kit.
- **Biological:** Environmental water samples may contain pathogens. Treat all samples as potentially infectious.
- **Physical:** Microplate wells are fragile. Handle plates carefully to avoid cracking wells or cross-contaminating adjacent wells.

5.1. Required PPE

- ☐ Safety glasses or goggles
- ☐ Lab coat
- ☐ Nitrile gloves (change between samples to prevent cross-contamination)

5.2. Emergency Procedures

For stop solution (acid) contact with skin or eyes, flush immediately with copious water for at least 15 minutes. Refer to [SOP-L003 Hazardous Materials Handling](#).

6. Equipment and Materials

6.1. Equipment

Item	Notes
Microplate reader (450 nm filter)	Absorbance mode; confirm filter is installed
Microplate washer (optional)	Manual wash with squeeze bottle is acceptable
Adjustable micropipettes (10–100 μL , 100–1000 μL)	Calibrated per manufacturer schedule
Multichannel micropipette (optional)	For 8- or 12-channel dispensing
Plate shaker or orbital incubator	Low speed (200–300 rpm)
Timer	
Incubator or water bath (if kit requires temperature control)	Some kits specify 25 °C or 37 °C

6.2. Consumables

Item	Notes
ELISA kit (analyte-specific)	See Section 6 for kit selection
Disposable pipette tips	Use fresh tips for every transfer
Deionized water (Type II or better)	For dilutions and wash steps
Absorbent paper towels	For blotting plates after washing
Aluminum foil	For covering plates during incubation

7. ELISA Kit Selection

The EA Program uses commercial competitive ELISA kits. The table below lists the kits currently validated for use. All kits follow the same general competitive ELISA workflow (Section 7) but differ in incubation times, dilution requirements, and detection ranges. Always consult the kit insert for analyte-specific instructions.

Table 1: ELISA kits validated or under evaluation for EA Program use.

Analyte	Manufacturer	Catalog No.	Detection Range	Sample Volume	Status
Bisphenol A	Abraxis	530701	0.05–10 $\mu\text{g/L}$	50 μL	Active
Bisphenol B	Abraxis	<i>TBD</i>	<i>TBD</i>	50 μL	<i>TBD</i>
Atrazine	Abraxis	500001	0.04–5.0 $\mu\text{g/L}$	50 μL	Active
Glyphosate	Abraxis	500086	0.075–4.0 $\mu\text{g/L}$	50 μL	<i>TBD</i>
2,4-D	Abraxis	500201	0.05–5.0 $\mu\text{g/L}$	50 μL	<i>TBD</i>

i Note

Catalog numbers and detection ranges are based on Abraxis (Eurofins) kits as of 2025. Verify against the current kit insert before use. Other manufacturers (EnviroLogix, Neogen) produce equivalent kits that may be substituted after validation.

8. Procedure

8.1. General Competitive ELISA Workflow

All competitive ELISA kits in this SOP share the same principle: the analyte in the sample competes with an enzyme-labeled analyte conjugate for a limited number of antibody binding sites coated on the microplate well. Higher analyte concentrations in the sample result in less conjugate binding and therefore lower color development after substrate addition. The relationship between absorbance and concentration is inversely proportional (Goda et al., 2000; Voller et al., 1978).

8.2. Preparation

1. Remove the ELISA kit from the refrigerator and allow all reagents to equilibrate to room temperature (20–25 °C) for at least 30 minutes before opening.
2. Prepare wash buffer by diluting the concentrate per kit instructions (typically 1:10 with deionized water). Mix thoroughly.
3. If samples require dilution (e.g., wastewater with expected high concentrations), prepare dilutions in deionized water or the sample diluent provided with the kit. Record all dilution factors.
4. Label a plate map in the lab notebook showing the position of standards, controls, blanks, and samples. Each standard and sample should be run in duplicate.

8.3. Assay Procedure

Follow the specific kit insert for exact volumes and incubation times. The general sequence is:

1. **Add standards and samples.** Pipette the specified volume (typically 50 μL) of each calibration standard, positive control, negative control (zero standard), and sample into the appropriate wells. Change tips between every addition.
2. **Add enzyme conjugate.** Pipette the conjugate solution into each well. The conjugate competes with any analyte present in the sample for antibody binding sites.
3. **Incubate.** Cover the plate with foil and incubate on the plate shaker at the specified temperature and duration (typically 60 minutes at room temperature for BPA; consult kit insert for other analytes).
4. **Wash.** Aspirate or decant the well contents. Wash each well 3–5 times with wash buffer (approximately 250 μL per wash). After the final wash, invert the plate and tap firmly on absorbent paper to remove residual liquid. Incomplete washing is the most common source of high background and poor precision.

5. **Add substrate.** Pipette the TMB substrate solution into each well. Incubate for the specified time (typically 20–30 minutes at room temperature) in the dark. A blue color develops in wells with bound conjugate.
6. **Add stop solution.** Pipette the stop solution (acid) into each well. The blue color turns yellow. Read the plate within 15 minutes of adding stop solution.
7. **Read absorbance.** Measure absorbance at 450 nm using the microplate reader.

 Tip

Dispense reagents quickly and consistently across the plate. Use a multichannel pipette when possible to minimize time differences between the first and last wells. Stagger plates if running more than one analyte simultaneously.

8.4. Plate Washing Details

Inadequate washing is the most frequent source of assay failure in ELISA. Follow these guidelines:

1. Aspirate well contents completely (manual flicking or vacuum aspiration)
2. Fill each well with approximately 250 μL wash buffer
3. Allow wash buffer to soak for 5–10 seconds
4. Aspirate again
5. Repeat for the total number of wash cycles specified by the kit (typically 3–5)
6. After the final aspirate, invert the plate and tap firmly on a pad of clean paper towels 3–4 times to remove residual buffer

9. Data Analysis and Calculations

9.1. Standard Curve Construction

1. Calculate the mean absorbance of the duplicate wells for each standard concentration.
2. Express each standard as a percentage of the zero standard (maximum binding, B_0):

$$\%B/B_0 = \frac{\bar{A}_{\text{standard or sample}}}{\bar{A}_{B_0}} \times 100$$

3. Plot $\%B/B_0$ (y-axis) against the log of the standard concentration (x-axis). Fit a four-parameter logistic (4PL) curve, which is the standard model for competitive immunoassay calibration (Findlay et al., 2000):

$$y = D + \frac{A - D}{1 + \left(\frac{x}{C}\right)^B}$$

where A = maximum asymptote (zero dose), D = minimum asymptote, $C = \text{IC}_{50}$ (inflection point), and B = slope factor.

- Interpolate sample concentrations from the 4PL curve. Most microplate reader software includes 4PL curve fitting. Alternatively, use R:

```
# Four-parameter logistic (4PL) curve fitting for ELISA data
library(drc)

# Example data: concentration (ug/L) and %B/B0
standards <- data.frame(
  conc = c(0.05, 0.1, 0.5, 1.0, 5.0, 10.0),
  pct_b_b0 = c(92.3, 84.1, 55.7, 38.2, 12.4, 6.8)
)

# Fit 4PL model
model_4pl <- drm(pct_b_b0 ~ conc, data = standards,
  fct = LL.4(names = c("Slope", "Lower", "Upper", "IC50")))
summary(model_4pl)

# Predict concentration for a sample with %B/B0 = 45.0
sample_pct <- 45.0
pred_conc <- ED(model_4pl, sample_pct, type = "absolute", display = FALSE)
cat(sprintf("Sample concentration: %.3f ug/L\n", pred_conc[1]))
```

9.2. Dilution Correction

If samples were diluted before analysis, multiply the interpolated concentration by the dilution factor:

$$C_{\text{original}} = C_{\text{interpolated}} \times DF$$

9.3. Reporting

Report results as $\mu\text{g/L}$ (ppb) for water samples. Indicate whether the result is a screening (ELISA) value and whether it exceeds any applicable regulatory threshold. Flag any result that falls outside the linear range of the standard curve (below the lowest standard or above the highest standard) and re-run with appropriate dilution if above range.

10. Quality Control

10.1. Within-Assay QC

- Run all standards and samples in duplicate. The coefficient of variation (CV) between duplicates should be less than 15%. If a duplicate pair exceeds 15% CV, re-run those samples.
- Include the kit-supplied positive control on every plate. The control must fall within the range stated on the certificate of analysis.

- Include at least one reagent blank (deionized water) per plate.
- The zero standard (B_0) absorbance should typically be greater than 0.8 (consult kit insert for acceptable range). Low B_0 values indicate degraded antibody or conjugate.

10.2. Between-Assay QC

- Run a laboratory-prepared quality control sample (spiked DI water at a known concentration within the linear range) with each batch. Recovery should be 80–120%.
- Track control chart performance (mean and ± 2 SD limits) over time.
- Record kit lot numbers and expiration dates. Do not mix reagents from different kit lots.

10.3. Method Detection Limits

The method detection limit (MDL) for each analyte is kit-specific and is defined as the concentration producing a $\%B/B_0$ response statistically distinguishable from the zero standard at the 95% confidence level. MDLs are typically provided in the kit insert. Report results below the MDL as “less than MDL.”

10.4. Cross-Reactivity

Competitive ELISA antibodies may cross-react with structurally related compounds. For example, BPA antibodies may exhibit partial cross-reactivity with BPB, BPS, or BPF. Atrazine antibodies may cross-react with simazine, propazine, or other triazine herbicides. Consult the cross-reactivity table in the kit insert and report results accordingly (e.g., “total triazines” rather than “atrazine” if significant cross-reactivity is known) (Aga, 2013).

11. Troubleshooting

Problem	Possible Cause	Corrective Action
High background (all wells yellow)	Incomplete washing; contaminated wash buffer	Increase wash cycles; prepare fresh buffer
Poor standard curve ($r^2 < 0.95$)	Reagent degradation; pipetting error	Check kit expiration; verify pipette calibration
Sample absorbance above B_0	Matrix interference; very low analyte	Dilute and re-run; consider sample cleanup
Sample absorbance below lowest standard	Concentration exceeds range	Dilute sample and re-run
High CV between duplicates ($> 15\%$)	Bubbles in wells; inconsistent pipetting	Tap plate to remove bubbles; use multichannel pipette
Positive control out of range	Kit degradation; incorrect storage	Check storage conditions; use new kit
Edge effects (outer wells differ)	Temperature gradient across plate	Use plate shaker; avoid outer wells for critical samples

12. Waste Disposal

Waste Type	Disposal Method
Used microplate and wells	Standard trash (after stop solution is added)
Stop solution waste (acid)	Neutralize with NaHCO ₃ ; drain with copious water
TMB substrate waste	Standard liquid waste; drain
Conjugate and antibody waste	Standard liquid waste; non-hazardous at kit dilutions
Sample waste	Drain with copious water (non-hazardous surface water)
Pipette tips	Standard trash

13. References

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14. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	Required safety training
L003	Hazardous Materials Handling	Waste and chemical safety
L303	Hach Colorimetric Analysis	Complementary screening for nutrients/metals
L308	Microplastics in Water	Related contaminant screening
L309	Pesticide Analysis (GC-MS/LC-MS)	Confirmatory instrumental method
F300	Surface Water Sampling	Sample collection context

15. Training Record

Before performing ELISA analyses independently, personnel must:

1. Read this SOP in full
2. Complete [SOP-L002 Laboratory Safety](#) training
3. Review the SDS for the ELISA kit reagents (especially stop solution)
4. Review the kit insert for the specific analyte(s) to be analyzed
5. Observe a trained operator perform a complete assay (plate setup through data analysis)
6. Perform the assay under supervision with acceptable QC results (duplicate CV less than 15%; positive control within range; standard curve $r^2 > 0.98$)
7. Be signed off by the PI or lab manager