Intended Use

The Beacon TPH 20 Tube Kit is an immunoassay for the detection of TPH in water samples including produced waters and hydraulic fracturing water. This product is intended for research use only.

Principles

Assay reagents are added to the test tubes to initiate a reaction. During an incubation, TPH in the calibrator/sample and TPH HRP Enzyme Conjugate compete for binding to the polyclonal TPH antibody immobilized on the test tubes surface. Following the incubation, the tubes are washed to remove any unbound TPH and TPH HRP Enzyme Conjugate. After washing, a colorless substrate is added to the tubes and any bound enzyme conjugate will convert the substrate to a blue color. Following an incubation, the reaction is stopped with the addition of Stop Solution and the amount of color in each tube is measured. The color of the unknown sample is compared to the color of the calibrators and the TPH concentration of the sample is derived.

Reagents and Materials Provided

1 Unit	Bag containing 20 test tubes that are vacuum sealed in an aluminized pouch with a desiccant.
1 Unit	Cylinder containing 10 capillary tubes.
1 Unit	Plunger.
1 Unit	Bag containing 2 zeroing tubes.
1 X 4 mL	Vial of Methanol.
4 X 2 mL	Vials of TPH Calibrators (Calibrator #1, Calibrator #2, Calibrator #3, Calibrator #4). Note: Calibrator reference concentration will vary based on sample matrix.
1 X 12 mL	Bottle of TPH HRP Enzyme Conjugate.
1 X 25 mL	Bottle of Assay Diluent.
1 X 14 mL	Bottle of Substrate.
1 X 14 mL	Bottle of Stop Solution.

Reagents and Materials Required but Not Provided

- Pipette(s) with disposable tips capable of dispensing the required volume(s).
- Repeater pipette(s) with disposable tips capable of dispensing the required volume(s) (recommended if running more than five tubes at once).
- Laboratory quality distilled or deionized water.
- Reagents and materials for sample collection, storage, and preparation.
- Personal protective equipment.
- Paper towels or equivalent absorbent material.
- Wash bottle (optional).
- Permanent marker.
- Tube rack.
- Timer.
- Photometer capable of reading absorbance at 450 nm with 12 mm x 75 mm tubes.

Kit Handling Notes and Precautions

- Read the product brochure in its entirety prior to use.
- The kit, in its original packaging, can be used until the end of the month indicated on the box label.
- Do not use reagents after their expiration date.
- Store all kit components at 2°C to 8°C (36°F to 46°F) when not in use.
- Reagents should be brought to room temperature, 20°C to 28°C (68°F to 82°F), prior to use. Avoid prolonged (> 24 hours) storage at room temperature.
- Do not freeze kit components or expose them to temperatures greater than 37°C (99°F).
- Running Calibrators and Samples in duplicate will improve assay precision and accuracy.
- Precise transfer of samples and reagents by using a calibrated pipette that is capable of dispensing the required volume is critical to obtain proper assay results.
- If running more than five tubes at once, the use of a repeater pipette is recommended when adding the Enzyme Conjugate, Substrate and Stop Solution.
- The plunger can be used many times however, the capillary tubes must be discarded after use. Be sure to use a clean capillary tube for each solution to avoid cross contamination. Alternatively, a positive displacement pipette with disposable tips capable of dispensing the required volume can be used to measure calibrators and samples.
- All procedural steps should be completed without interruption. Ensure all reagents, materials and equipment are ready at the appropriate time.
- Each reagent is optimized for use in the Beacon TPH 20 Tube Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon TPH 20 Tube Kits with different lot numbers.
- Do not reuse test tubes.
- Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.
- Damage to or obstruction of the optical surface may cause unsatisfactory results.

Interfering Substances

Substance	Interference Level	Recommended Solution
Chlorine (water samples only)	> 2 ppm	Add 1 drop of 0.1N sodium thiosulfate per 100 mL of sample.

Sample Collection, Storage, and Preparation

- Collect samples in glass or PTFE containers. Clean the containers with soap and water, then rinse the containers with methanol. Use PTFE-lined caps. If PTFE-lined caps are not available, use aluminum foil as a substitute cap liner. Be sure to rinse the aluminum foil with methanol prior to use. Completely fill the container and immediately tighten the cap.
- For the best results, analyze the samples as soon as possible after collection. If sample storage is necessary, put the sample in an ice bath or refrigerator to limit the loss of volatile compounds and use within 24 hours.
- No sample preparation is required for water samples. Water samples can be used directly in the assay.

Use of the Wiretrol Pipette

1. Insert the plunger into the colored end of the capillary tube. The unit is ready for use when the tip of the plunger slightly extends beyond the end of the capillary tube.
2. Insert the tip below the surface of the liquid. Aspirate the liquid by slowly and smoothly pulling the plunger upwards until the bottom of the plunger tip reaches the desired volume line. Touch the tip of the capillary tube to the side of the vessel to release any drops that may remain.
3. To dispense the liquid, insert the tip of the capillary tube below the surface of the receiving solution and apply a slow, even pressure downward until all liquid has been released.
4. Discard the capillary tube after use. Be sure to use a clean capillary tube for each solution to avoid cross contamination. The plunger can be thoroughly cleaned and reused many times.

Assay Procedure

1. Allow kit components and the sample(s) to reach room temperature prior to running the test.
2. Place the appropriate number of test tubes into a tube rack. Label the tubes one inch from the top with the calibrator or sample identification. Be sure to re-seal unused tubes in the zip-lock bag with the desiccant to limit exposure to moisture.
3. Dispense **500 µL of Assay Diluent** into each calibrator tube using a pipette.
4. Dispense **500 µL of each Sample** into the appropriate sample tube using a pipette. Be sure to use a clean pipette tip for each solution to avoid cross contamination.
5. Dispense **50 µL of each Calibrator** into the appropriate calibrator tube using the Wiretrol pipette. Be sure to use a clean capillary tube for each solution to avoid cross contamination. Mix the contents of each tube after addition.
6. Dispense **50 µL of Methanol** into each **sample tube** using the Wiretrol pipette. Be sure to use a clean capillary tube for each solution to avoid cross contamination. Mix the contents of each tube after addition.
7. Dispense **500 µL of Enzyme Conjugate** into each tube using a pipette.
8. Start a timer for **10 minutes**. Immediately, shake the tubes for 30 seconds using a gentle back-and-forth motion and incubate at room temperature. Halfway through the incubation, mix the contents of the tubes again using the same mixing procedure.
9. Decant the contents of the tubes into an appropriate waste container. Fill the tubes to overflowing with laboratory quality distilled or deionized water and then decant. Repeat this wash step three times for a total of four washes. Following the last wash, tap the inverted tubes onto absorbent paper to remove excess wash solution.
10. Dispense **500 µL of Substrate** into each tube using a pipette.
11. Start a timer for **10 minutes**. Immediately, shake the tubes for 30 seconds using a gentle back-and-forth motion and incubate at room temperature. Halfway through the incubation, mix the contents of the tubes again using the same mixing procedure.
12. Dispense **500 µL of Stop Solution** into each tube using a pipette in the same order of addition as the Substrate.
13. Gently shake the tubes for 20 seconds using a back-and-forth motion.
14. Carefully wipe the optical surface with a soft, lint-free wipe. Measure and record the absorbance (Optical Density; OD) of each tube at 450 nm using a tube reader within 10 minutes of stopping the assay. Be sure to blank the reader using a zeroing tube filled with laboratory quality distilled or deionized water prior to measuring.
15. Dispose of used test tubes in an appropriate waste container.

Quality Control (QC) Criteria

- The correlation coefficient (R^2) of the calibration curve, analyzed using a 4-parameter logistic regression, must be ≥ 0.99.
- The average absorbance of the zero calibrator replicates must be ≥ 0.2.
- The average absorbance of calibrator replicates must have a coefficient of variation (%CV) < 15%.
- The average absorbance of sample replicates must have a coefficient of variation (%CV) < 20%.

Result Interpretation

- Determine the calibrator concentrations:
  The TPH calibrators are formulated to show a known concentration of diesel fuel. Refer to the matrix-specific tables below to use calibrators for other TPH compounds.

Compound	Calibrator #1 (ppm)	Calibrator #2 (ppm)	Calibrator #3 (ppm)	Calibrator #4 (ppm)
Diesel Fuel	2	5	10	20
Gasoline	1.5	3.5	4	14
Kerosene	3.5	7.5	14	24
Benzene	2	4.5	8.5	16
Toluene	1.5	3	5	9
Ethylbenzene	0.5	1.5	3.5	7.5
m-Xylene	0.9	2	3.5	7
o-Xylene	1	2	4	8
p-Xylene	0.3	0.5	0.9	16
BTEX	0.5	1.5	2.5	4.5

- Graph the data using a 4-parameter curve fit. Alternatively, a semi-log curve fit can be used if 4-parameter is not available.
- Ensure QC criteria are met.
- Interpret the results as follows:
  - Samples with a lower absorbance (less color) than a calibrator have a concentration of TPH greater than the concentration of the calibrator.
  - Samples with a higher absorbance (more color) than a calibrator have a concentration less than the concentration of the calibrator.
  - Samples with absorbances greater than the lowest calibrator or less than the highest calibrator must be reported as < Calibrator #1's concentration (ppm) or > Calibrator #4's concentration (ppm), respectively.
- In the event that the water sample contains a concentration greater than that of Calibrator #4, further dilute the sample in laboratory quality distilled or deionized water and retest alongside the calibrators. Sample results must be multiplied by the total dilution factor used. The recommended dilution protocol is detailed below:

  1. Measure either 0.5, 1, 2, 5, 10, or 25 mL of sample into a graduated cylinder capable of holding 50 mL.
  2. Add laboratory quality distilled or deionized water to the graduated cylinder to the 50 mL mark.
  3. Follow the assay procedure to analyze the diluted sample.
  4. When interpreting the results, multiply the calibrator concentrations by the appropriate multiplier in accordance with the table below:

Sample Volume (mL)	Multiplier
0.5	100
1	50
2	25
5	10
10	5
25	2

Technical Assistance
For questions regarding this kit or for additional information about Beacon products, contact us.

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Safety
Stop Solution is 1N hydrochloric acid. Handle with care. To receive complete safety information on this product, contact Beacon Analytical Systems, Inc., and request Safety Data Sheets.

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