

Universal Nucleic Acid ELISA Kit

For Laboratory Use

Catalog Number: EL2054

For Research Use Only. Not for use in Diagnostic Procedures.

I. Introduction

Attogene's universal ELISA assay kit is a ready-to-use, universal anti-FITC/FAM (EL2054-01) or anti-Dig (EL2054-02) coated 96 well ELISA plate to conveniently capture dual labeled nucleic acid molecules tagged with biotin. The test is designed to conveniently develop qualitative or quantitative rapid test systems for detection of nucleic acids with detection reagent HRP conjugated streptavidin.

The detection of nucleic acid (DNA or RNA) using this system requires the use of a biotin and fluorescein isothiocyanate or 6-carboxyfluorescein (FITC/FAM)-labelled nucleic acid for the EL2054-01 kit version, nucleic acids containing biotin and digoxigenin (dig) for the EL2054-02-kit version. In creating nucleic acids using amplification, the primers should be designed so that the final product contains both moieties on the final product. We have optimized the plate coating with the optimal antibody concentration and variant to provide maximal performance for nucleic acid based ELISA applications.

The sample containing the nucleic acids to be detected is simply mixed into the specially designed nuclease free sample dilution buffer in a well of the supplied 96-well plate, mixed and the detection reagent is then added. Generally, the test procedure is completed in 60 minutes. It is important to note that the relative stoichiometry between the nucleic acid added, and the detection reagent is important in assay optimization. Using nucleic acid products in excess of the test's dynamic detection range will lead to inaccurate results.

We've established a dynamic detection range with FITC/FAM to be in the range of 10-760 attomoles/uL of sample. Dig has a dynamic detection range of 10-920 attomoles/uL. While this may be a safe range to start with, running a set of standards with a known concentration of the analyte is recommended as variability may arise with different user samples.

Features & Benefits

- Can be used for the development of an ELISA assay for detection of a variety of different molecules such as amplified DNA products from PCR, LAMP and RPA reactions.
- Can be used in DNA-binding protein assays, hybridization-based detection, molecular inhibition screening, and nuclease activity testing.
- No expensive equipment required
- Cost-effective way to screen for further downstream assay development.

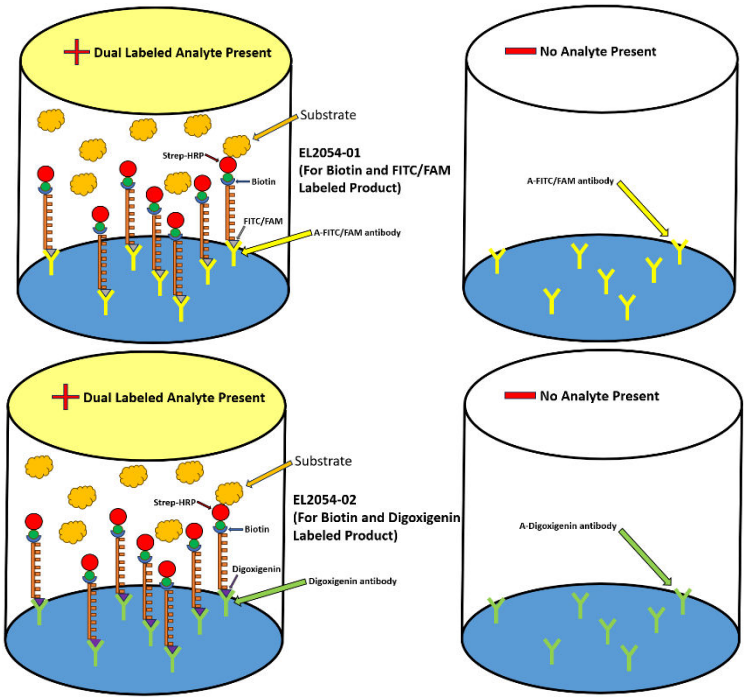


Figure 1. Schematic representation of assay mechanism in the presence and absence of a dual-labeled analyte after the introduction of the stop-solution.

2. Kit Contents

Component Name	Volume	Storage
Anti-FITC/FAM (EL2054-01) or Anti-Dig (EL2054-02) Coated 96-well Plate	1 each	Refrigerate
ELISA 20X Wash Solution	28 mL	Refrigerate
Control Nucleic Acid Standards 0 amol/uL green cap, 180 amol/uL purple cap, 300 amol/uL yellow cap, 540 amol/uL blue cap, 640 amol/uL orange cap, 760 amol/uL red cap containing biotin and FAM (EL2054-01 kit only)	(6X vials 800uL/vial)	Refrigerate

Component Name	Volume	Storage
Control Nucleic Acid Standards 0 amol/uL green cap, 160 amol/uL purple cap, 340 amol/uL yellow cap, 520 amol/uL blue cap, 700 amol/uL orange cap, 920 amol/uL red cap containing biotin and DIG (EL2054-02 kit only)	(6X vials 800uL/vial)	Refrigerate
100X Detection Reagent (EL2054-01/02)	250uL	Refrigerate
Detection Reagent Dilution Buffer	12mL	Refrigerate
Nuclease Free Sample Diluent	28mL	Refrigerate
TMB Substrate Solution	12mL	Refrigerate
Stop Solution	12mL	Refrigerate
Manual	1 each	RT

3. Storage and Stability

- The kit should be stored at 2°C - 4°C until ready to use.
- The antibody coated plates must remain in the desiccated bag until use.

4. Required Materials Not Supplied

5.1 Equipment

- ELISA reader 450nm/630nm (ELISA Plate Readers are available on Attogene.com)
- Vortex mixer
- Timer
- Wash bottle
- Micropipettes:20μl-200μl, 100μl-1000μl, 300μl-multipipette

5. Precautions

- Prior to use, ensure that the product has not expired by verifying that the date of use is prior to the expiration date on the label.
- The plates are packaged in a desiccated bag. Do not remove unless they are to be used subsequently for testing.

- Avoid cross-contamination of samples by using a new tube and disposable pipette tip for each sample.
- Use only ELISA Kit reagents from one kit lot, as they have been adjusted in combination.
- It is good laboratory practice to use positive and negative controls to ensure proper test performance.
- If no signal is detected in the well, the sample may be too diluted, or the analyte's labeling may be insufficient/defective.
- If multiple sample dilutions show absorbance values similar to the 50fmol/50uL standard (red cap), it may be necessary to dilute the sample further to bring it into the assay's dynamic range.
- Please use one tip in the process of experiment and change the tips when absorbing different reagents.
- If running more than two rows at once, the use of a multichannel pipette is required.
- Make sure that all experimental instruments are clean.

6. Reagent Preparation

- If your sample is too high of a concentration, dilute to the appropriate range of concentrations using the nuclease free sample dilution buffer.
- 1X Wash solution: combine one volume of the 20X Wash Solution with 19 volumes of deionized water. Mix well.
- 1X HRP-conjugated Detection Reagent: combine one volume of the 100X HRP-Conjugated Detection Reagent with 99 volumes of Detection Reagent Diluent. Vortex for 10 seconds to mix.

Prepare this solution fresh before each test.

7. Procedure

Instructions prior to beginning the assay.

- Ensure that all reagents and microwells are at room temperature (20-28°C).
- Return all reagents to 2-8°C immediately after their use.
- Wash the microwells correctly; this is a vital factor in the reproducibility of the ELISA analysis.
- Avoid direct sunlight during the incubation.

Steps in the assay process.

- Remove all reagents from cold storage and leave out at room temperature (20-28°C) for at least 30min. Shake gently before use.
- Collect the microwells necessary for the assay and return the rest into the bag at 2-8°C immediately.
- The diluted wash solution should be brought to room temperature before use.
- Number every microwell position. All standards and samples should be run in duplicate. Record the standards and samples positions.
- Dispense **50µL of the Dual Labeled Nucleic Acid standards, negative control, or sample** into each well.
- Dispense **100µL of Detection Reagent** into appropriate test wells.
- Shake the plate gently for 30 seconds using a back-and-forth motion.
- Cover the plate. Incubate for **30 minutes** at room temperature.
- Decant the contents of the wells into an appropriate waste container.
- Rinse the microwells with 250µL of the 1X Wash Solution 3 times.
- Absorb the residual wash solution in the well by wrapping the plate securely in absorbent paper and inducing rapid acceleration/deceleration of the plate on a solid surface. Ensure wells are secure prior to carrying out this procedure.
- Add **100µL TMB Substrate Solution** to each well, mix gently by shaking the plate manually and incubate for **15min** at RT with plate covered.
- Add **100µL of Stop Solution** to each well. Mix gently by shaking the plate manually and measure the absorbance at 450nm (Read the result within 5min after addition of Stop Solution).

8. Interpretation of Results

This test is an ELISA assay where the absorbance is dependent on the concentration of the dual labeled nucleic acid. Higher concentrations of the analyte will produce higher absorbance values.

Calculating the Percentage Absorbance

- The mean values of the absorbance values obtained from the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%.

Absorbance (%) = B / B_0 *100

B = the mean absorbance value of each standard or each sample

B_0 = absorbance value of zero standard

Drawing a Standard Curve:

- To draw a standard curve, the absorbance value of standards as y-axis, the concentration of the standards (ppb) as x-axis is adjusted to be semilogarithmic.
- The concentration of each sample (ppb), which can be read from the standard curve, is multiplied by the corresponding dilution factor of each sample followed, and the actual concentration of sample is obtained.

9. General Instructions

Temperature of reagents and samples:

- The mean values of the absorbance values obtained for the standards and the samples will be reduced if the reagents and samples have not been regulated to room temperature (20-25°C).

Microwells:

- Do not allow microwells to dry between steps to avoid unsuccessful reproducibility and operate the next step immediately after tapping the microwells holder.

Shaking of reagents:

- Shake each reagent gently before use.

Skin protection:

- The Stop Solution is 0.75N HCl, keep your skin away from it.

Out-of-date kits:

- Don't use kits that are expired. Don't exchange the reagents of different batches, or else it will impact validity/efficacy of the assay.

Who we are:

Attogene is a biotechnology company located in Austin, Texas. Our focus is to enhance health and wellness by offering and developing customer focused Life Science Products domestically and internationally.

Our mission is to:

- Enhance detection technologies
- Enable rapid responses
- Enable impactful research discoveries

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