Epigenase™ APOBEC3A Activity/Inhibition Assay Kit

Base Catalog # P-3142

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The Epigenase™ APOBEC3A Activity/Inhibition Assay Kit is suitable for measuring the activity/inhibition of APOBEC3A enzyme using cell extracts or purified APOBEC3A from human and other mammals, in a variety of forms including, but not limited to cultured cells, fresh and frozen tissues.

Starting Materials: Input materials can be cell extracts or purified APOBEC3A enzymes. The amount of cell extracts for each assay can be 2 µg to 30 µg with an optimal range of 15-20 µg. The amount of purified enzymes can be 10 ng to 1 µg with an optimal range of 50 ng to 200 ng, depending on the type, purity, and catalytic activity of the enzymes.

Internal Control: The APOBEC3A assay standard is provided in this kit for the quantification of APOBEC3A enzyme activity. Because APOBEC3A activity can vary from tissue to tissue and from normal and diseased states, it is advised to run replicate samples to ensure that the signal generated is validated.

Precautions: To avoid cross-contamination, carefully pipette the sample or solution into the strip wells. Use aerosol-barrier pipette tips and always change pipette tips between liquid transfers. Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.

110 Bi County Blvd. Ste. 122, Farmingdale, NY 11735
Tel: 1-877-374-4368 ■ Fax: 1-718-484-3956 ■ E-mail: info@epigentek.com ■ Web: www.epigentek.com

KIT CONTENTS

Component	48 Assays Cat. #P-3142-48	96 Assays Cat. #P-3142-96	Storage Upon Receipt
WB (10X Wash Buffer)	14 ml	28 ml	4°C
A3AB (APOBEC3A Assay Buffer)	3 ml	6 ml	4°C
A3AS (10X APOBEC3A Substrate)*	10 µl	20 µl	–20°C
BS (Binding Solution)	5 ml	10 ml	RT
AAS (APOBEC3A Assay Standard, 20 µg/ml)*	10 µl	20 µl	–20°C
CA (Capture Antibody, 1000 µg/ml)*	5 µl	10 µl	4°C
DA (Detection Antibody, 400 µg/ml)*	6 µl	12 µl	–20°C
ES (Enhancer Solution)*	5 µl	10 µl	–20°C
DS (Developer Solution)	5 ml	10 ml	4°C
SS (Stop Solution)	5 ml	10 ml	RT
8-Well Assay Strips (With Frame)	6	12	4°C

** For maximum recovery of the products, centrifuge the original vial prior to opening the cap.*

SHIPPING & STORAGE

The kit is shipped in two parts: the first part at ambient room temperature, and the second part on frozen ice packs at 4°C. Upon receipt: (1) store **A3AS**, **AAS**, **DA**, and **ES,** at –20°C away from light; (2) store **WB**, **A3AB**, **CA**, **DS**, and **8-Well Assay Strips** at 4°C away from light; (3) store remaining components (**BS** and **SS**) at room temperature away from light.

***Note**: Check if **WB** (10X Wash Buffer) contains salt precipitates before use. If so, warm at room temperature or 37°C and shake the buffer until the salts are re-dissolved.*

All components of the kit are stable for 6 months from the date of shipment, when stored properly.

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Adjustable pipette or multiple-channel pipette
- ☐ Multiple-channel pipette reservoirs
- ☐ Aerosol resistant pipette tips
- ☐ Microplate reader capable of reading absorbance at 450 nm
- ☐ 1.5 ml microcentrifuge tubes
- ☐ Incubator for 37°C incubation
- ☐ Distilled water
- ☐ Cell extract or purified enzymes
- ☐ Parafilm M or aluminum foil

GENERAL PRODUCT INFORMATION

Quality Control: Each lot of Epigenase™ APOBEC3A Activity/Inhibition Assay Kit is tested against predetermined specifications to ensure consistent product quality. EpigenTek guarantees the performance of all products in the manner described in our product instructions.

Product Warranty: If this product does not meet your expectations, simply contact our technical support unit or your regional distributor. We also encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

Safety: Suitable lab coat, disposable gloves, and proper eye protection are required when working with this product.

Product Updates: EpigenTek reserves the right to change or modify any product to enhance its performance and design. The information in this User Guide is subject to change at any time without notice. Be sure to use the latest User Guide for this kit which can be accessed online at www.epigentek.com/datasheet.

Usage Limitation: The Epigenase™ APOBEC3A Activity/Inhibition Assay Kit is for research use only and is not intended for diagnostic or therapeutic application

Intellectual Property: The Epigenase™ APOBEC3A Activity/Inhibition Assay Kit and methods of use contain proprietary technologies by EpigenTek.

A BRIEF OVERVIEW

The APOBEC3A cytosine deaminase (Apolipoprotein B mRNA-editing enzyme catalytic polypeptide 3A) is a DNA/RNA editing enzyme. APOBEC3A is found exclusively in mammals and can catalyze the removal of an amino group from the cytosine (C) and 5-methylcytosine (5-mC) base to form uridine (U) and thymidine, respectively, in single-strand DNA or RNA. The genetic alterations by APOBEC3A will change the transcription and mRNA processing and actively participate in various biological processes.

APOBEC3A is a part of the innate immune system against various DNA or RNA viruses to protect the integrity of cells. Through promoting the deamination of cytosine to uracil on ssDNA/RNA, APOBEC3A generates mutations in virus genomes to inhibit virus replication. For example, a C-to-U mutation caused by APOBEC3s in SARS-CoV-2 genomes was reported to be the dominant mutation (>50%). APOBEC3A was also found to be among the most abundant mRNA in COVID-19 patients, which probably inhibits SARS-CoV-2 replication through damaging the virus-repairing enzyme nsp14. While APOBEC3A confers its intrinsic host resistance to infection of various viruses, including the SARS-CoV-2 virus, it may also cause potential evolutionary consequences for viruses.

APOBEC3A contributes significantly to DNA mutagenesis in cancer. As a major driver of cancer evolution, APOBEC3A plays an important role in genomic mutations detected in a variety of tumors. APOBEC3 mutation signatures can be seen in more than 50% of various cancers, promoting carcinogenesis that furthers cancer progression and resistance to therapies. In addition, APOBEC3A is the only APOBEC3 enzyme that can efficiently deaminate 5-methylcytidine to thymidine. It participates (alone or combined with methylated DNA/RNA oxidative enzymes such as TETs) in local DNA demethylation to support epigenetic cellular identity and regulation.

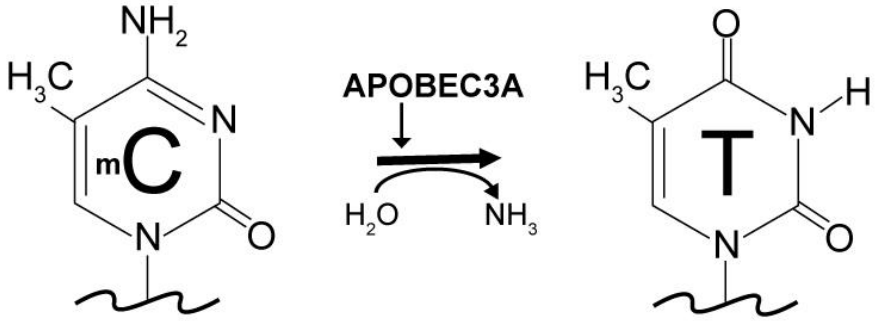

Fig 1. Methylcytosine deamination reaction catalyzed by APOBEC3A

Thus, detection of APOBEC3A enzyme activity and inhibition would be important in benefiting virus infection control, cancer diagnostics, and developing new target-based cancer therapeutics. Currently, there are no/few methods for detecting APOBEC3A activity using cell extracts from cells or tissues. To address this issue, EpigenTek developed and offers the Epigenase™ APOBEC3A Activity/Inhibition Assay Kit. The kit has the following advantages and features:

- Colorimetric assay with easy-to-follow steps that can be finished within 5 hours.
- Innovative kit composition enables background signals to be extremely low, allowing the assay to be simple, accurate, reliable, and consistent.
- Both cell/tissue extracts and purified APOBEC3A enzymes can be used for detecting APOBEC3A inhibitor effects *in vivo* and *in vitro*.
- Novel assay principle allows for high sensitivity with a detection limit as low as 10 ng of purified APOBEC3A enzymes.
- The assay standard is included for quantifying the specific activity of APOBEC3A.
- Strip microplate format for manual or high throughput analysis (96 assays).

PRINCIPLE & PROCEDURE

The Epigenase™ APOBEC3A Activity/Inhibition Assay Kit is designed for measuring APOBEC3A activity/inhibition. In an assay with this kit, the unique 5mC substrate is stably coated on the strip wells. Active APOBEC3A binds to and converts 5mC contained in the substrate to T. The un-converted 5mC in the substrate can be recognized with a high-affinity 5mC antibody, and the immuno-signal is improved with enhancer solution. The ratio or amount of un-converted 5mC, which is inversely proportional to enzyme activity, can then be colorimetrically quantified through an ELISA-like reaction.

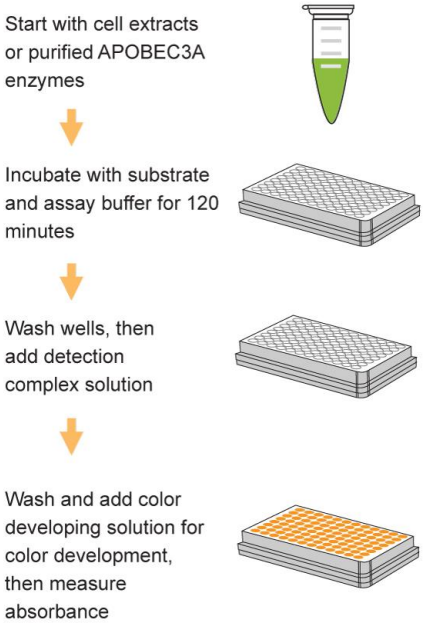

Fig. 2. Schematic procedure of Epigenase™ APOBEC3A Activity/Inhibition Assay Kit.

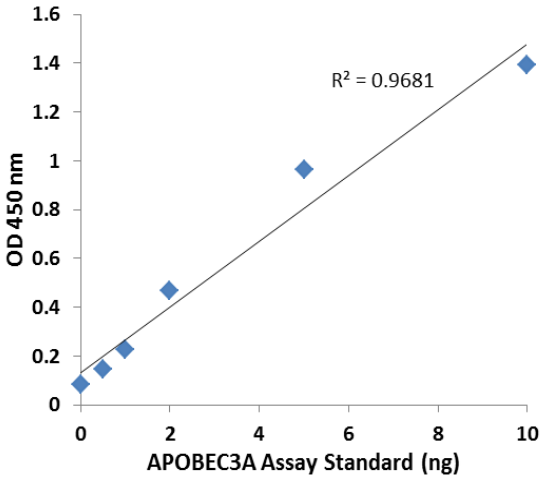

Fig. 3. Illustrated standard curve generated with the APOBEC3A assay standard.

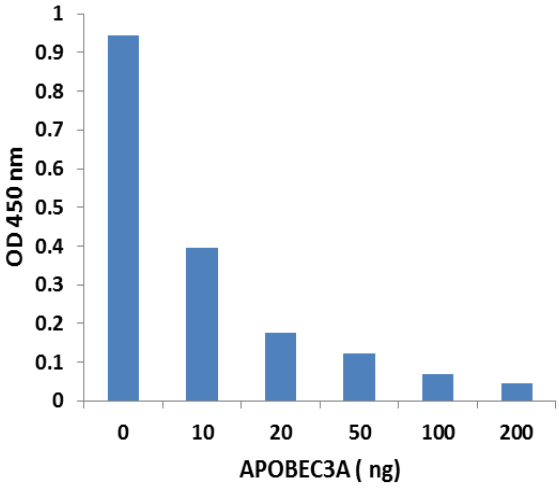

Fig. 4. APOBEC3A activity achieved by using recombinant APOBEC3A with the Epigenase™ APOBEC3A Activity/Inhibition Assay Kit. Recombinant human APOBEC3A enzyme was added at different concentrations. OD is inversely proportional to APOBEC3A enzyme activity.

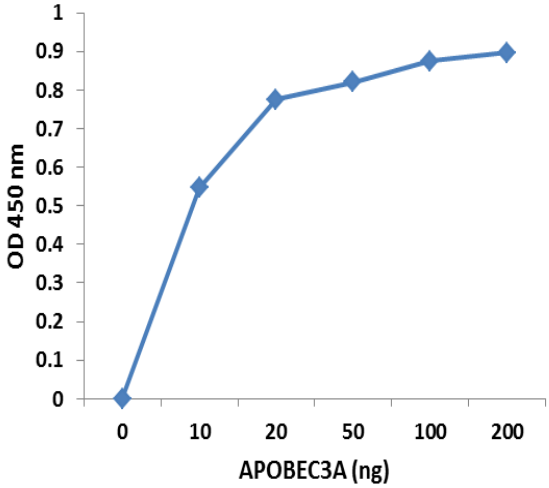

Fig. 5. Converted OD from raw OD data shown in Fig.4.

ASSAY PROTOCOL

For the best results, please read the protocol in its entirety prior to starting your experiment.

Starting Materials

Input Amount: The amount of cell extracts for each assay can be 2 µg to 30 µg with an optimal range of 15-20 µg. The amount of purified enzymes can be 10 ng to 1 µg with an optimal range of 50 ng to 200 ng, depending on the purity and catalytic activity of the enzymes.

Cell Extraction: You can use your method of choice for preparing cell extracts. EpigenTek offers a whole cell extraction kit (Cat # OP-0003) optimized for use with this kit.

Storage of Cell Extract or Purified APOBEC3A Proteins: Using freshly prepared cell extract is the best. Otherwise, the cell extract or purified APOBEC3A enzymes should be stored in aliquots at –80°C until use.

1. Buffer Solution & Preparation

a. Prepare **Diluted WB** (1X Wash Buffer):

48-Assay Kit: Add 13 ml of **WB** (10X Wash Buffer) to 117 ml of distilled water and adjust pH to 7.2-7.5.

96-Assay Kit: Add 26 ml of **WB** (10X Wash Buffer) to 234 ml of distilled water and adjust pH to 7.2-7.5.

This **Diluted WB** (1X Wash Buffer) can now be stored at 4°C for up to six months.

b. Prepare **1X A3AS** (1X APOBEC3A Substrate):

Add 1 µl of **A3AS** (10X APOBEC3A Substrate) to each 9 µl of **BS** (Binding Solution). About 2 µl of **1X A3AS** will be required for each assay well.

c. Prepare **Diluted AAS** (Assay Standard):

Suggested Standard Curve Preparation: First, dilute **AAS** (APOBEC3A Assay Standard) with **BS** (Binding Solution) to 10 ng/µl by adding 5 µl of **AAS** to 5 µl of **BS**. Then, further prepare five concentrations by combining the 10 ng/µl **Diluted AAS** with **BS** into final concentrations of 0.20, 0.5, 1, 2, 5, and 10 ng/µl according to the following dilution chart:

Tube	AAS (10 ng/µl)	BS	Resulting Diluted AAS Concentration
1	1.0 µl	49.0 µl	0.20 ng/µl
2	1.0 µl	19.0 µl	0.5 ng/µl
3	1.0 µl	9.0 µl	1 ng/µl
4	1.0 µl	4.0 µl	2 ng/µl
5	2.0 µl	2.0 µl	5 ng/µl
6	4.0 µl	0.0 µl	10 ng/µl

***Note**: Keep each of diluted solutions except **Diluted WB** (1X Wash Buffer) on ice until use. Any remaining diluted solutions other than **Diluted WB** should be discarded if not used within the same day.*

2. Enzymatic Reaction

a. Predetermine the number of strip wells required for your experiment. Carefully remove un-needed strip wells from the plate frame and place them back in the bag (seal the bag tightly and store at 4°C).

b. Add 80 µl of **BS (**Binding Solution) to each well.

c. Add 2 µl of **1X A3AS** (1X APOBEC3A Substrate) into each sample well and control wells without cell extracts or purified enzymes. Add 2 µl of **A3AB** (APOBEC3A Assay Buffer) into blank wells. Add 2 µl of **Diluted AAS** (Assay Standard) into the standard curve wells (see the designated wells depicted in Table 1 under "Suggested Strip Well Setup" below). Mix solution by gently tilting from side to side or shaking the plate several times. Ensure the solution coats the bottom of the wells evenly.

***Note**: For the standard curve, add 2 µl of **Diluted AAS** (Assay Standard) at concentrations of 0.0 to 10 ng/µl (see the chart in Step 1c). The final concentrations should be 0.4 1, 2, 4, 10, and 20 ng per well.*

d. Cover strip plate with plate seal or Parafilm M and incubate at 37°C for 90 min.

e. Remove the **BS** (Binding Solution) from each well.

f. Wash each well three times with 150 µl of the **Diluted WB** (1X Wash Buffer) each time.

g. Blank Wells: Add 50 µl of **A3AB** (APOBEC3A Assay Buffer) to each blank well.

h. Standard Wells: Add 50 µl of **A3AB** (APOBEC3A Assay Buffer) to each standard well.

i. Control Wells without Cell Extracts or Enzymes: Add 45 µl of **A3AB** (APOBEC3A Assay Buffer) and 5 µl of your protein extraction buffer.

j. Sample Wells Without Inhibitor: Add 45 µl of **A3AB** (APOBEC3A Assay Buffer) and 5 µl of cell extracts or purified enzyme to each sample well without inhibitor. Total volume should be 50 µl per well.

k. Sample Wells With Inhibitor: Add 40 µl of **A3AB** (APOBEC3A Assay Buffer), 5 µl of cell extracts or purified enzyme, and 5 µl of inhibitor solution. Total volume should be 50 µl per well.

***Note**: (1) Follow the suggested well setup diagrams under "Suggested Strip Well Setup"; (2) The concentration of inhibitor to be added into the sample wells can be varied (1 µM to 1000 µM). However, the final concentration of the inhibitors before adding to the wells should be prepared with **A3AB** (APOBEC3A Assay Buffer) at a 1:10 ratio (i.e., add 0.5 µl of inhibitor to 4.5 µl of **A3AB**) so that the original solvent of the inhibitor can be reduced to 1% of the reaction solution or less.*

l. Tightly cover strip plate with **Parafilm M** to avoid evaporation and incubate at 37°C for 120 min.

***Note**: The incubation time may depend on intrinsic APOBEC3A activity. However, in general, 120 min incubation is suitable for active purified APOBEC3A enzyme.*

m. During the last 10 minutes of sample incubation, prepare the **Detection Complex Solution**: In each 1 ml of **Diluted WB** (1X Wash Buffer) add 1 µl of **CA**, (Capture Antibody) mix and then add 1 µl of **DA** (Detection Antibody) and 0.5 µl of **ES** (Enhancer Solution). Mix well.

n. Remove the reaction solution from each well. Wash each well three times with 150 µl of the **Diluted WB** (1X Wash Buffer) each time.

3. Signal Detection

a. Add 50 µl of the **Detection Complex Solution** to each well, then cover and incubate at room temperature for 50 minutes.

b. Remove the **Detection Complex Solution** from each well.

c. Wash each well with 150 µl of the **Diluted WB** (1X Wash Buffer) for a total of five times each.

Note: *Ensure any residual wash buffer in the wells is thoroughly removed at each wash step. The wash can be carried out by simply pipetting the wash buffer into the wells and then pipetting the buffer out from the wells (discard the buffer).*

d. Add 100 µl of **DS** (Developer Solution) to each well and incubate at room temperature for 1 to 10 min away from light. Begin monitoring color changes in the sample wells and control wells. The **DS** solution will turn blue in the presence of sufficient un-converted substrate.

e. Add 100 µl of **SS** (Stop Solution) to each well to stop enzyme reaction when the color in the positive control wells turns medium blue. The color will change to yellow after adding **SS** and the absorbance should be read on a microplate reader within 2 to 10 min at 450 nm with an optional reference wavelength of 655 nm.

Note: *(1) Most microplate readers have the capability to carry out dual-wavelength analysis and will automatically subtract reference wavelength absorbance from the test wavelength absorbance. If your plate reader does not have this capability, the plate can be read twice – once at 450 nm and once at 655 nm. Then manually subtract the 655 nm ODs from 450 nm ODs; (2) If the stripwell microplate frame does not fit in the microplate reader, transfer the solution to a standard 96-well microplate.*

4. APOBEC3A Activity Calculation

a. Calculate the average for duplicate readings for sample wells and blank wells.

b. Calculate enzyme activity or inhibition using the following formulas:

For simple calculation:

$$\text{APOBEC3A activity (OD/h/mg)} = \frac{[\text{OD (control}^{\#} - \text{blank}) - \text{OD (sample} - \text{blank})]}{[\text{Protein Amount (µg)}/1000]^{*} \times \text{Hour}^{**}}$$

Control wells without cell extracts or purified enzymes
* Protein amount added into the reaction at Step 2j.
** Incubation time at Step 2l (in hours).

Example calculation:

Average OD450 of sample is 0.25
Average OD450 of blank is 0.075
Average OD450 of control is 0.5
Protein amount is 5 µg
Incubation time is 2 h

$$\text{APOBEC3A Activity} = \frac{(0.5 - 0.25)}{(5 \times 2)} \times 1000 = 18 \text{ OD/h/mg}$$

For accurate or specific activity calculation:

First, generate a standard curve and plot the OD values versus the amount of **AAS** at each concentration point. Then determine the slope as OD/ng using linear regression (*Microsoft Excel's* linear regression or slope functions are suitable for such calculation) and the most linear part (include at least 4 concentration points) of the standard curve for optimal slope calculation. Now calculate APOBEC3A activity using the following formula:

$$\text{Activity (ng/h/mg)} = \frac{[\text{OD (control}^{\#} - \text{blank}) - \text{OD (sample} - \text{blank})]}{\text{Slope} \times \text{Protein Amount (µg)}^{*} \times \text{Hour}^{**}} \times 1000$$

Control wells without cell extracts
* Protein amount added into the reaction at Step 2j
** Incubation time at Step 2l (in hours).

For inhibition calculation:

$$\text{Inhibition \%} = \left(1 - \frac{[\text{OD (control} - \text{blank}) - \text{OD (inhibitor sample} - \text{blank})]}{[\text{OD (control} - \text{blank}) - \text{OD (no inhibitor sample} - \text{blank})]}\right) \times 100\%$$

SUGGESTED STRIP WELL SETUP

Table 1. Approximate amount of required buffers and solutions for defined assay wells based on the protocol.

Reagents	1 well	1 strip (8 wells)	2 strips (16 wells)	6 strips (48 wells)	12 strips (96 wells)
Diluted WB	2.5 ml	20 ml	40 ml	120 ml	240 ml
A3AB	50 µl	400 µl	800 µl	2400 µl	4800 µl
1X A3AS	2 µl	16 µl	32 µl	96 µl	192 µl
AAS	N/A	N/A	4 µl (optional)	8 µl	8 µl
BS	80 µl	640 µl	1080 µl	3840 µl	7680 µl
Diluted CA	50 µl	400 µl	800 µl	2400 µl	4800 µl
Diluted DA	50 µl	400 µl	800 µl	2400 µl	4800 µl
Diluted ES	50 µl	400 µl	800 µl	2400 µl	4800 µl
DS	0.1 ml	0.8 ml	1.6 ml	4.8 ml	9.6 ml
SS	0.1 ml	0.8 ml	1.6 ml	4.8 ml	9.6 ml

Table 2. The suggested strip-well plate setup for standard curve preparation in a 48-assay format (in a 96-assay format, Strips 7 to 12 can be configured as Sample). The controls and samples should be measured in duplicate.

Well #	Strip 1	Strip 2	Strip 3	Strip 4	Strip 5	Strip 6
A	Blank	Blank	Sample	Sample	Sample	Sample
B	AS 0.20 ng/µl	AS 0.20 ng/µl	Sample	Sample	Sample	Sample
C	AS 0.5 ng/µl	AS 0.5 ng/µl	Sample	Sample	Sample	Sample
D	AS 1 ng/µl	AS 1 ng/µl	Sample	Sample	Sample	Sample
E	AS 2 ng/µl	AS 2 ng/µl	Sample	Sample	Sample	Sample
F	AS 5 ng/µl	AS 5 ng/µl	Sample	Sample	Sample	Sample
G	AS 10 ng/µl	AS 10 ng/µl	Sample	Sample	Sample	Sample
H	No sample Control	No sample Control	Sample	Sample	Sample	Sample

TROUBLESHOOTING

Problem	Possible Cause	Suggestion
No signal or weak signal in both the standard and sample wells	Reagents are added incorrectly.	Check if reagents are added in the proper order with the right amount, and if any steps in the protocol may have been omitted by mistake.
	The substrate and standard are not properly bound to the wells.	Ensure that (1) the **A3AS** (10X APOBEC3A Substrate) and **AAS** (APOBEC3A Assay Standard) are added into the wells; (2) the wells are completely covered with sufficient **BS** (Binding Solution); and (3) binding time is sufficient (90 min).
	Incubation time and temperature are incorrect.	Ensure that the incubation time and temperature described in the protocol are followed correctly.
	Incorrect absorbance reading.	Check if the appropriate absorbance wavelength (450 nm filter) is used.
	Kit was not stored or handled properly.	Ensure all components of the kit were stored at the appropriate temperatures and the cap is tightly secure after each opening or use.
No signal or weak signal in only the standard curve wells	The standard amount is insufficiently added to the well in Step 2c.	Ensure a sufficient amount of standard is added.
	The standard is degraded due to improper storage conditions.	Follow the storage guidance in the User Guide for **AAS** (APOBEC3A Assay Standard).

High background present in the blank wells	Insufficient washing of wells.	Check if washing at each step is performed according to the protocol.
	Contaminated by sample or standard.	Ensure the well is not contaminated from adding sample or standard accidentally or from using contaminated tips.
	Incubation time with Detection Complex Solution is too long.	The incubation time at Step 3a should not exceed 60 minutes.
	Over development of color.	Decrease the development time in Step 3d before adding **SS** (Stop Solution) in Step 3e.
No signal or weak signal only in sample wells	Protein sample is not properly extracted or purified.	Ensure your protocol is suitable for DNA demethylase extraction. For the best results, use EpigenTek's Whole Cell Extraction Kit (Cat. No. OP-0003). Also, use fresh cells or tissues for protein extraction, as frozen cells or tissues could lose enzyme activity.
	Sample amount added into the wells is insufficient.	Ensure a sufficient amount of cell extracts is used as indicated in Steps 2j and 2k. The sample can be titrated to determine the optimal amount to use in the assay.
	Sample was not stored properly or has been stored for too long.	Ensure the sample is stored in aliquots at –80°C, with no more than 6 weeks for cell extracts. Avoid repeated freezing/thawing.
	Little or no activity of APOBEC3A contained in the sample.	This problem may be a result of many factors. If the affecting factors cannot be determined, use new/re-prepared cell extracts or active APOBEC3A enzymes.
Uneven color development	Insufficient washing of the wells.	Ensure the wells are washed according to the user guide. Ensure residual wash buffer is removed as much as possible.
	Delayed color development or delayed stopping of color development in the wells.	Ensure color development and stop solutions are sequentially added and consistent with the order you added the other reagents (e.g., from well A to H or from well 1 to 12).

RELATED PRODUCTS

Nuclear Extract Preparation

OP-0003 EpiQuik™ Whole Cell Extraction Kit

DNA Demethylase

P-3086 Epigenase™ 5mC-Hydroxylase TET Activity/Inhibition Assay Kit (Colorimetric)

P-3094 Epigenase™ Thymine DNA Glycosylase (TDG) Activity/Inhibition Assay Kit (Colorimetric)