Human p16INK4a ELISA Kit
Sandwich ELISA
Catalog EL-213-1616

This kit employs a sandwich enzyme immunoassay technique to quantify p16INK4a (CDKN2A) in biological samples.

INTRODUCTION

CDKN2A is a locus that, through use of alternate first exons, gives rise to more than one distinct protein, the best known of which is p16INK4a. p16INK4a works as a brake on cell division by physically attaching to the cyclin-dependent kinases CDK4 and CDK6, blocking them from teaming up with cyclin D and thereby stopping them from inactivating the retinoblastoma protein, a step normally required for a cell to pass from G1 into S phase. Its levels climb as cells accumulate DNA damage or oncogenic stress, making p16INK4a one of the principal drivers that pushes a cell into permanent growth arrest, or senescence, rather than continued division, and it is used as a molecular marker of that senescent state and of tissue aging more broadly. Because this brake is so central to preventing uncontrolled proliferation, the CDKN2A locus is one of the most frequently deleted, mutated, or silenced regions in human cancer, and inherited defects in it underlie hereditary melanoma and related melanoma-pancreatic cancer predisposition syndromes.

PRINCIPLE OF THE ASSAY

This sandwich enzyme immunoassay utilizes a microtiter plate pre-coated with a capture antibody specific to human p16INK4a (CDKN2A). During the first incubation, p16INK4a present in standards or samples is captured by this immobilized antibody. A biotin-conjugated secondary antibody, also specific to human p16INK4a, is then added and binds a distinct epitope on the captured analyte, forming a sandwich complex. Avidin conjugated to horseradish peroxidase (HRP) is subsequently added to each well, where it binds the biotin moiety. Following addition of TMB substrate, only wells containing the complete sandwich complex — captured p16INK4a, biotin-conjugated detection antibody, and avidin-HRP — develop a colorimetric signal. The enzymatic reaction is terminated by addition of a sulphuric acid stop solution, and absorbance is measured at 450 nm (± 10 nm). p16INK4a concentrations in unknown samples are determined by interpolating their absorbance values against a standard curve.

MATERIALS AND STORAGE

Store kit components at -20°C unless specified otherwise. DO NOT USE past kit expiration date. Some vials contain a small amount of reagents. Spin tubes on pulse setting prior to opening.

Each kit includes:	Units
Pre-Coated Microplate	12 strips x 8 wells
Standard (Lyophilized)	2 vials
Biotinylated Antibody (100×)	120 µL
Streptavidin-HRP (100×)	120 µL
Standard/Sample Diluent Buffer	20 mL
Biotinylated Antibody Diluent	12 mL
HRP Diluent	12 mL
Wash Buffer (25×)	20 mL
TMB Substrate Solution	10 mL
Stop Reagent	6 mL
Plate Covers	2 pieces
Do not mix or substitute reagents with those from other lots.	

MATERIALS AND INSTRUMENTS REQUIRED BUT NOT SUPPLIED

- Precision pipettes calibrated to deliver 5-1000µL
- Multi-channel pipette calibrated to deliver 50-200µL
- Plate shaker
- Disposable tips
- Vortex-Mixer
- Distilled or de-ionized water
- Microplate reader capable of reading 450nm with background subtraction at 620nm

SAFETY PRECAUTIONS

- The test protocol must be followed strictly.
- All reagents containing human material should be handled as if potentially infectious. Operators should wear gloves and protective

clothing when handling any patient sera or serum based products.

- The kit reagents contain antimicrobial agents, acid and 3,3’,5,5’-tetramethylbenzidine. Avoid contact with the skin and eyes. Rinse immediately with plenty of water if any contact occurs.
- Any liquid that has been brought into contact with potentially infectious material has to be discarded in a container with a disinfectant. Disposal must be performed in accordance with local regulations.
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- Only trained laboratory personnel should execute this test.

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PREPARATION OF REAGENTS

Prepare only the appropriate amount of required reagent on the day of use. Store all reagents as per instructions stated on the label.

1. **Wash Buffer (1X) Preparation: Dilute the 25X wash buffer concentrate 1:25 with double-distilled Water.**
2. **Detection Reagent (1X) Preparation: Dilute the biotinylated antibody and streptavidin-HRP concentrates 100-fold with Biotinylated Antibody Diluent and HRP Diluent, respectively, immediately before use.**
3. **Preparation of Calibrators: Prepare calibrators by serial dilution from a 40 down to 0.63 (plus a 0 blank), per the source manual.**

Sol'n ID	Source	Source Vol (µL)	Cal. Diluent (µL)	Final Vol (µL)	Final Conc. (ng/mL)
Std 1	Stock Standard	500	500	1000	40
Std 2	Std 1	500	500	1000	20
Std 3	Std 2	500	500	1000	10
Std 4	Std 3	500	500	1000	5
Std 5	Std 4	500	500	1000	2.5
Std 6	Std 5	500	500	1000	1.25
Std 7	Std 6	500	500	1000	0.63
Blank	Std 7	500	500	1000	0

SPECIMEN STORAGE

This kit is compatible with EDTA-plasma, heparin-plasma and serum samples. Samples can be stored at or below -20°C for up to 1 year.

ASSAY PROCEDURE

1. After the kit is equilibrated at room temperature, add 100 µL of Standard Working Buffer (gradually diluted according to the instructions) or 100 µL of sample to each well, and incubate at 37°C for 80 minutes.

2. Discard the liquid in the plate, add 200 µL 1× Wash Buffer to each well, and wash the plate 3 times. After pat it dry against clean absorbent paper, add 100 µL Biotinylated Antibody Working Solution (1×) to each well, incubate at 37°C for 50 minutes.

3. Discard the liquid in the plate, add 200 µL 1× Wash Buffer to each well, and wash the plate 3 times. After pat it dry against clean absorbent paper, add 100 µL 1× Streptavidin-HRP Working Solution to each well, incubate at 37°C for 50 minutes.

4. Discard the liquid in the plate, add 200 µL 1× Wash Buffer to each well, and wash the plate 5 times. After pat it dry against clean absorbent paper, add 90 µL TMB Substrate Solution to each well, incubate at 37°C for 20 minutes in the dark.

5. Add 50 µL Stop Solution to each well, shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm immediately, calculation of the results.

CALCULATIONS AND RESULTS

1. Construct a standard curve by plotting the absorbance obtained from each standard against concentration. Use a 4 or 5 parameter curve fit. Alternatively a log-log curve fit may be used.
2. The concentration of the unknowns can be read directly from this standard curve using the absorbance value for each sample.

3. Any sample undiluted or diluted still reading greater than the highest standard should be diluted appropriately and retested. If the samples have been diluted, the concentration determined from the standard curve must be multiplied by the dilution factor.

PERFORMANCE CHARACTERISTICS

Precision: Intra-assay CV < 8%. Inter-assay CV < 10%.

Detection Limit: 0.267 ng/mL

Recovery: Serum (n = 5): 80-97% (avg 88%); EDTA plasma (n = 5): 77-95% (avg 86%); Heparin plasma (n = 5): 94-106% (avg 100%)

Specificity: This assay has high sensitivity and excellent specificity for detection of Human CDKN2A. No significant cross-reactivity or interference between Human CDKN2A and analogues was observed.

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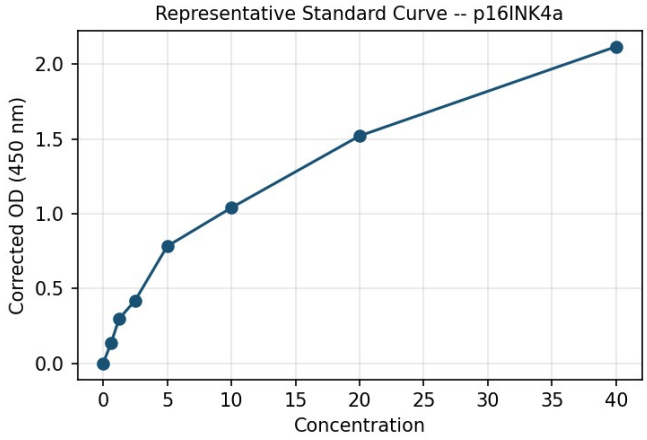
SAMPLE STANDARD CURVE



Representative standard curve shown for illustration only. This curve should not be used to calculate unknown sample concentrations -- run a new standard curve with each assay.

ORDERING INFORMATION

Please visit *www.affinityimmuno.com* to order this product. Visa, Mastercard, AMEX and PayPal are accepted in our online store.

Your order will be processed immediately and you will be notified with a delivery timeframe.