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## Datasheet for ABIN3073432 hCG ELISA Kit

### Overview

Quantity:	96 tests
Target:	hCG
Binding Specificity:	intact
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	10-1000 MioU/mL
Minimum Detection Limit:	10 MioU/mL
Application:	ELISA

### Product Details

Purpose:	The Total hCG enzyme linked immunosorbent assay (ELISA) kit provides materials for the quantitative measurement of Total hCG in biological fluids.
Sample Type:	Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Components:	<ul style="list-style-type: none"><li>• Total hCG Calibrator A-F</li><li>• Total hCG Controls</li><li>• Anti-Total hCG Antibody Coated Microtitration strips</li><li>• Total hCG Assay Buffer</li><li>• Total hCG Antibody-Enzyme Conjugate Ready-to-Use</li><li>• Extraction Buffer</li><li>• TMB Chromogen Solution</li></ul>

## Product Details

- Wash Concentrate A
- Stopping Solution

Material not included:	<ol style="list-style-type: none"><li>1. Microtitration plate reader capable of absorbance measurement at 450 nm, 405nm and 630 nm.</li><li>2. Microtitration orbital plate shaker.</li><li>3. Microtitration plate washer.</li><li>4. Semi-automated/manual precision pipette to deliver 2-250 µL.</li><li>5. Vortex mixer.</li><li>6. Deionized water.</li><li>7. Disposable 12 x 75 mm culture tubes.</li><li>8. Tight fitting 12 x 75 mm tube racks.</li></ol>
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## Target Details

Target:	hCG
Alternative Name:	hCG ( <a href="#">hCG Products</a> )

## Application Details

Sample Volume:	25 µL
Assay Time:	1 h
Plate:	Pre-coated
Reagent Preparation:	<ol style="list-style-type: none"><li>1. Wash Solution: Dilute wash concentrate 25-fold with deionized water. The wash solution is stable for one month at room temperature (~25 °C) when stored in a tightly sealed bottle.</li><li>2. Microtitration Wells: Select the number of coated wells required for the assay. The remaining unused wells should be placed in the resealable pouch with a desiccant. The pouch must be resealed to protect from moisture.</li></ol>
Sample Collection:	<ul style="list-style-type: none"><li>• Serum is the recommended sample type.</li><li>• Use the following recommendations for handling, processing and storing blood samples.<sup>2</sup></li><li>• Allow samples to clot for two hours at room temperature or overnight at 4°C and follow blood collection tube manufacturer's recommendations for centrifugation. Keep tubes stoppered at all times. Within two hours after centrifugation, transfer at least 500 µL of cell free sample to a storage tube. Tightly stopper the tube immediately.<ul style="list-style-type: none"><li>◦ Samples if used within 24 hours may be stored at 4 °C, otherwise samples must be stored at -20 °C or -80 °C to avoid loss of bioactivity and contamination.</li><li>◦ Remove residual fibrin and cellular matter prior to analysis.</li><li>◦ Avoid assaying lipemic, hemolyzed or icteric samples</li><li>◦ Each laboratory should determine the acceptability of its own blood collection tubes and</li></ul></li></ul>

serum separation products.

- Avoid repeated freezing and thawing of samples. Thaw samples no more than 3 times.

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### Assay Procedure:

Allow all samples and reagents to reach room temperature (~25 °C). Mix reagents thoroughly by gentle inversion before use. After reconstitution of reagents, mix thoroughly, avoiding foam. Calibrators, controls and samples should be assayed in duplicate.

1. Mark the microtitration strips to be used.
2. Pipet 10 µL of the calibrators, controls and unknown blood spot extracted samples to the appropriate wells.
3. Add 200 µL of the Total hCG Assay Buffer to each well using a precision pipette.
4. Incubate the wells, shaking at 600-800 rpm on an orbital microplate shaker, for 30 mins at room temperature (~25 °C).
5. Aspirate and wash each well 5 times with the wash solution using an of automatic microplate washer or manually using a precision pipette. NOTE: Use an automatic microplate washer is strongly recommended. Incomplete washing will adversely affect assay precision. If a microplate washer is not available to wash the plate, manually: a.) Completely aspirate the liquid from each well b.) Dispense 300 µL of the wash solution into each well using a precision pipette c.) Aspirate the liquid again d.) Repeat steps (b) and (c) 5 times
6. Add 100 µL of the antibody-enzyme conjugate ready-to-use solution to each well using a precision pipette.
7. Incubate the wells, shaking at 600-800 rpm on an orbital microplate shaker, for 30 mins at room temperature (~25 °C).
8. Aspirate and wash each well 5 times with the wash solution using an automatic microplate washer.
9. Add 100 µL of the TMB chromogen solution to each well using a precision pipette. Avoid exposure to direct sunlight.
10. Incubate the wells, shaking at 600-800 rpm on an orbital microplate shaker, for 8-12 min at room temperature (~25 °C). NOTE: Please be aware that the color may develop more quickly or more slowly than the recommended incubation time depending on the localized room temperature. Please visually monitor the color development to optimize the incubation time.
11. Add 100 µL of the stopping solution to each well using a precision pipette.
12. Read the absorbance of the solution in the wells within 20 minutes, using a microplate reader set to 450 nm. NOTE: • While reading the absorbance of the microtitration well, it is necessary to program the zero calibrator as a "Blank". • If wavelength correction is available, set the instrument to dual set wavelength measurement at 450 nm with background wavelength correction between 600 and 630 nm.

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### Calculation of Results:

NOTE: The results in this package insert were calculated by plotting the data on a log vs. log scale using a linear curve-fit. Other data reduction methods may give slightly different results.

1. Calculate the mean absorbance for each calibrator, control or sample. Using data reduction software set the program to use log-log scaling with 4PL plotting the mean absorbance readings for each of the calibrators along the y-axis versus the Total hCG concentrations in

## Application Details

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mIU/mL on the x- axis.

2. Determine the Total hCG concentrations of the controls and samples from the calibration curve by matching their mean absorbance readings with the corresponding Total hCG concentrations.
3. Any sample reading higher than the highest calibrator should be appropriately diluted using Extraction Buffer and reassayed.
4. Any sample reading lower than the analytical sensitivity should be reported as such.
5. Convert the blood spot extract values to serum equivalent levels in mIU/mL by multiplying the measured concentrations in IU/ by the approximate dilution factor of the extraction method (~250) and then divide the sum by
6. NOTE: If the absorbance readings exceed the limitations of the plate reader, a second reading at 405 nm is needed (reference filter between 600 and 630 nm if available). In this case, proceed to construct a second calibration curve as above with the absorbance readings of all calibrators at 405 nm. The concentration of the off-scale samples at 450 nm is then read from the new calibration curve. The readings at 405 nm should not replace the on-scale readings at 450 nm.

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Restrictions:	For Research Use only
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## Handling

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Precaution of Use:	For Research Use Only. The following precautions should be observed: a) Follow good laboratory practice. b) Use personal protective equipment. Wear lab coats and disposable gloves when handling immunoassay materials. c) Handle and dispose of all reagents and material in compliance with applicable regulations WARNING: Potential Biohazardous Material This reagent may contain some human source material (e.g. serum) or materials used in conjunction with human source materials. Handle all reagents and patient samples at a Biosafety Level 2, as recommended for any potentially infectious human material in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 5th Edition, 2007. WARNING: Potential Chemical Hazard Some reagents in this kit contain Pro-Clean 400 as a preservative. Pro-Clean 400 and peroxide in concentrated amounts are irritants to skin and mucous membranes. For further information regarding hazardous substances in the kit, please refer to the MSDS.
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Storage:	4 °C
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