REAGENTS

The **ASI ProPhase Plus** test cassette contains a goat polyclonal antibody-coated membrane and a pad containing mouse monoclonal IgG-dye conjugate in a protein matrix containing sodium azide as a preservative.

WARNINGS AND PRECAUTIONS

For *In Vitro* Diagnostic Use

1. Human urine should be handled as if capable of transmitting infectious agents. The CDC/NIH Health Manual "Biosafety in Microbiological Laboratories" describes how these materials should be handled in accordance with Good Laboratory Practice.
2. Do not pipet by mouth.
3. Do not smoke, eat, drink or apply cosmetics in areas where patient samples are handled.
4. Any cuts, abrasions or other skin lesions should be suitably protected.
5. Dispose of all used test components in a proper biohazard container.

Handling and Procedural Notes

1. In order to obtain reliable and consistent results, the instructions in the package insert must be strictly followed. Do not modify the handling and storage conditions for the cassette or samples.
2. Do not use past the expiration date indicated on the cassette pouch.

Storage Instructions

Store the kit contents at 2-28° C (36-82° F); do not freeze. Test cassettes must be at room temperature for use.

Indications of Deterioration

Do not use the test cassette if the protective pouch has been punctured, if the device appears damaged, or if the membrane appears discolored or damaged.

SPECIMEN COLLECTION AND STORAGE

1. Urine specimens must be collected in clean glass, plastic, or wax-coated containers free of preservatives.
2. Urine collected anytime may be used; however, the first morning urine usually contains the highest concentration of hCG.
3. If the sample is not to be tested immediately following collection, but is to be tested within 48 hours after collection, the sample should be refrigerated (2-8° C; 36-46° F). It must be brought back to room temperature (15-30° C; 59-86° F) before testing.
4. If a sample will not be tested until more than 48 hours after collection, it should be stored frozen (-20° C; -4° F or below) for not more than two weeks. Prior to testing, frozen samples must be completely thawed, thoroughly mixed, and brought to room temperature (15-30° C; 59-86° F).

PERFORMANCE OF THE TEST

Materials Provided

	25 Tests	375 Tests
Foil Pouch, Containing One Each Of: ProPhase Test Cassette Single-Use Dropper Moisture Absorbent Packet	25	15 x 25
Package Insert	1	15 x 1

Additional Materials Required

1. Timing device
2. Specimen collection container

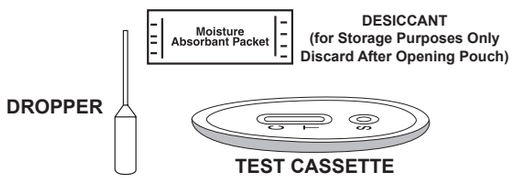
TEST PROCEDURE

Preparation for the Assay

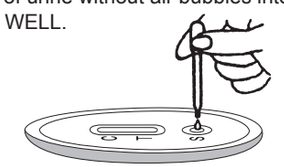
1. Carefully read the instructions before beginning the test. Do not open the foil pouch until ready to perform the test.
2. Allow the patient sample and any controls to come to room temperature (15-30° C; 59-86° F).

ASSAY PROTOCOL

1. Open a foil pouch by tearing at the notch and remove the TEST CASSETTE and DROPPER. Place the TEST CASSETTE on a clean, level surface.



2. Fill the DROPPER with urine and hold it vertically above the SAMPLE WELL (marked "S"). Dispense four (4) drops of urine without air bubbles into the round SAMPLE WELL.



3. Read the result after five (5) minutes, then discard the CASSETTE. Do not read a test result after more than five minutes.

NOTE: When performing **ASI ProPhase Plus** hCG testing, if a pink-rose color migration is not observed within five (5) minutes after the addition of the four (4) drops of urine to the SAMPLE WELL in the TEST CASSETTE, add an additional one (1) or two (2) drops to the SAMPLE WELL and read the result five (5) minutes after this second urine addition.

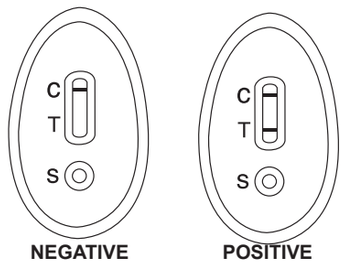
Quality Control

The use of positive and negative controls is recommended to insure proper kit performance. Quality control samples should be tested according to quality control requirements established by the testing laboratory, following the procedures described in this insert.

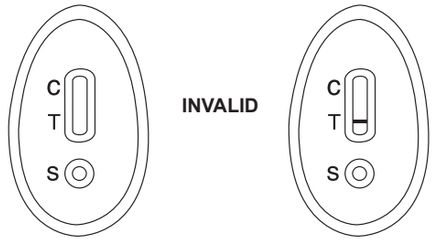
Each **ASI ProPhase Plus** test cassette contains a built-in quality control indicator which causes a pink-rose color line to appear in the control zone, marked "C", if the test is functioning correctly. If no colored line appears in the control zone on the test strip, the test did not function properly.

INTERPRETATION OF RESULTS

1. **Negative:** One pink-rose band appears in the control zone marked "C", with no band in the test zone marked "T". A negative result indicates that the concentration of hCG is below the detection sensitivity of the test and that the patient is not pregnant or it is too soon for the pregnancy to be detected.
2. **Positive:** Two pink-rose bands appear, one in the test zone marked "T" and one in the control zone marked "C". A positive result indicates that hCG has been detected at or above a concentration of 25 mIU/ml in the sample, a strong indicator that the patient is pregnant. The colored bands may vary in intensity.



3. **Invalid:** If no pink-rose bands are visible, or if a band is visible only in the test zone marked "T" and not in the control zone marked "C", then the result is invalid. An invalid result may be due to deterioration of the test reagents or to improper testing procedure. Carefully review the procedure and retest with a new cassette. Colored lines that appear after five (5) minutes are not diagnostic and should be ignored.



COMMON QUESTIONS

1. Q. Within seconds after the urine sample is added, if a pink color covers the entire window region, and there are a few vertical lines that appear darker than the rest of the window, does this indicate a positive test?
   A. After the urine sample is added, you will see a pink liquid movement that starts from the bottom and gradually moves toward the top of the control zone of the cassette. You may see a few vertical streaks; however, this is completely normal. You should never use any vertical streaks as part of your interpretation of the test result; only horizontal bands can be considered. The appearance of horizontal rose-pink bands across both window regions indicates a positive result.
2. Q. If exactly four (4) drops of urine are not used to perform the test, will it still give an accurate result?
   A. Four (4) drops is the recommended sample size. However, as you can see from the note following the procedure description, five (5) or even six (6) drops can be tolerated without compromising the test results. If less than four (4) drops are added, the test may not work; this can easily happen if the user is not careful to eliminate air bubbles from the dropper before delivery of the urine into the sample well of the cassette.
3. Q. After a certain length of time can the test result change?
   A. A positive result will not change for several days after the test is completed. However, some reddish background might be noticed several hours after the test is performed. A negative result should not be read in the test zone more than five (5) minutes after the test is performed. After 30 minutes, some negative tests might even appear to be weakly positive. This happens because the test chemicals keep reacting after the test is completed.
4. Q. How accurate is the test?
   A. There is a clinical data summary under the "Accuracy" section of this package insert; but it stops short of claiming 100% accuracy despite the fact that 100% is implied by the data, which contains

no discrepancies. A claim of 100% accuracy cannot be made due to the potential for user error and other such error sources. It is acceptable to claim that the test is "over 99% accurate."

LIMITATIONS OF THE PROCEDURE

1. In addition to pregnancy, hCG has been detected in patients with both gestational and non-gestational trophoblastic disease. These diagnoses should be ruled out in the interpretation of hCG levels to establish a diagnosis of pregnancy.
2. A normal pregnancy cannot be distinguished from an ectopic pregnancy based on hCG levels alone. Also, a spontaneous miscarriage may cause confusion in interpreting the test results.
3. A negative result from urine specimen collected from a woman in very early pregnancy may be due to an unusually low concentration of hCG. In such cases, the test should be repeated on a fresh specimen obtained approximately two days later.
4. A negative result may be obtained from a urine sample that is too dilute (does not contain an adequate concentration of hCG). If pregnancy is still suspected, obtain a first morning urine specimen and retest.
5. Although the **ASI ProPhase Plus** test is very accurate in detecting pregnancy, a low incidence of false results can occur. If results are unexpected or inconsistent, consult with a physician.
6. As with all diagnostic tests, a definitive diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

EXPECTED VALUES

Urine hCG levels are estimated to be (1-3):

1) 10-30 mIU/ml seven to ten days post-conception.
2) 37,000-50,000 mIU/ml eight to eleven weeks after last menstrual period.
3) Undetectable in healthy men or healthy nonpregnant women.

PERFORMANCE CHARACTERISTICS

SENSITIVITY

The **ASI ProPhase Plus** test will detect hCG in urine at a concentration of 25 mIU/ml or greater. This sensitivity has been confirmed with hCG standards (25, 50, 100, 1000, 450,000, and 900,000 mIU/ml) in urine, calibrated against WHO 1st IRP. Occasionally, specimens containing less than 25 mIU/ml hCG can also give positive results.

SPECIFICITY

Glycoprotein hormones which are potentially cross-reactive with anti-hCG present in the test were added to hCG-free, 25 mIU/ml hCG, and 450,000 mIU/ml hCG urine samples in the following concentrations:

1. LH - 50, 100, 300, and 500 mIU/ml
2. FSH - 50, 100, 500, and 1000 mIU/ml
3. TSH - 100, 500, and 1000 mIU/ml

The **ASI ProPhase Plus** test was used to assay each preparation. In all cases, the expected results were obtained and no cross-reactivity or interference was detected.

INTERFERING SUBSTANCES

The following potentially interfering substances were added to urine specimens containing hCG levels of 0 and 25 mIU/ml:

Substance	Concentration
Acetaminophen	20 mg/dl
Acetylsalicylic acid	20 mg/dl
Albumin	2000 mg/dl
Ampicillin	20 mg/dl
Ascorbic acid	20 mg/dl
Atropine	20 mg/dl
Caffeine	20 mg/dl
Cortisol	200 ng/ml
DHEAS	500 ng/ml
Estradiol (E-2)	25 ng/ml
Estriol (E-3)	25 ng/ml
Gentisic acid	20 mg/dl
Glucose	2000 mg/dl
Human serum protein	2000 mg/dl
Tetracycline	20 mg/dl
Uric acid	10 mg/dl

The **ASI ProPhase Plus** test was used to assay each preparation. In all cases, the expected results were obtained and none of the substances at the concentration tested interfered in the assay.

ACCURACY

A study was performed using a total of 190 positive and negative urine specimens. These specimens were randomly selected and assayed with the **ASI ProPhase Plus** test and another commercially available hCG test according to the respective package inserts.

ProPhase Plus

Other Test	Positive	Negative
Positive	102	0
Negative	0	84

Four specimens were weakly reactive with both tests and were not included in the data above. These specimen reactions were considered inconclusive. When the four specimens were retested in a quantitative radioimmunoassay (RIA), the results indicated that the samples contained between 20 and 35 mIU/ml hCG.

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WARRANTY

This product is warranted to perform as described in its labeling and ARLINGTON SCIENTIFIC, INC. literature. ARLINGTON SCIENTIFIC, INC. disclaims any implied warranty of merchantability or fitness for a particular purpose and in no event shall ARLINGTON SCIENTIFIC, INC. be liable for consequential damages.

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 ZL-2111 10/03

ASI PROPHASE PLUS™

One-Step hCG Test

For *in vitro* diagnostic use

Cat. No.	ZL-2110	25 Tests (Kit)
	ZL-2210	375 Tests (Case)

INTENDED USE

ASI ProPhase Plus is a single-step immunoassay for the qualitative determination of human chorionic gonadotropin (hCG) in urine for the early detection of pregnancy.

SUMMARY AND EXPLANATION

Human chorionic gonadotropin is a glycopeptide hormone produced by the placenta beginning shortly after fertilization. In normal pregnancy, hCG can be detected in serum and urine as early as 7 days following conception (1-5). At the time of the first missed menstrual period, hCG levels of 100 mIU/ml may be detected in the maternal urine, and peak levels are seen late in the first trimester of pregnancy (1-9). The early appearance of hCG in urine following conception has made it the marker of choice for the early detection of pregnancy.

ASI ProPhase Plus is a single-step immunochromatographic assay which utilizes a combination of monoclonal and polyclonal antibodies to detect hCG with a high degree of specificity and sensitivity. The presence of hCG in maternal urine may be detected at the time of the first missed menses without interference from other hormones.

PRINCIPLE OF THE PROCEDURE

The immunochromatographic cassette device contains a unique set of dye-conjugated and immobilized antibodies, which produces a distinctive visual pattern in the result window when the hCG concentration of the test sample is 25 mIU/ml or greater. An elevated hCG concentration is detected in approximately five minutes.

Urine sample migrates through the absorbent area mixing with labeled antibody-dye conjugate; hCG present in the specimen binds to the conjugate forming an antibody-antigen complex. As the reaction mixture flows through the test zone "T", the complex binds to immobilized anti-hCG, producing a pink-rose color band. The appearance of the color band in the test zone indicates that hCG is present at or above the cutoff sensitivity of 25 mIU/ml. In the control zone "C", unbound conjugate binds to immobilized reagents producing a pink-rose color band. The appearance of this band indicates that the test is functioning properly.

The **ASI ProPhase Plus** test is standardized against the World Health Organization First International Reference Preparation (WHO 1st IRP).