# SELF-STIK®

## Test Strips

### WARNINGS AND PRECAUTIONS

SELF-STIK Reagent Strips are for *in vitro* diagnostic use and are intended for professional use only. The "universal precautions" recommended by the Centers for Disease Control should be adhered to whenever blood or body fluids are handled. These precautions include wearing gloves.

SELF-STIK urine test strips may contain either diazonium salt or nitroferricyanide. Avoid contact with skin and mucous membranes; flush affected areas with copious amounts of water. Get immediate medical attention for eyes or if ingested. Exercise the normal precautions required for handling all laboratory reagents.

### SUMMARY AND INTENDED USE

SELF-STIK reagent strip is a dip and read test strip and intended for use as an in vitro diagnostic aid using urine specimens. The strip contains solid phase reagent areas affixed to a plastic support and is provided in a dry reagent format. This strip is a visual qualitative and semi-quantitative test for the determination of blood, bilirubin, urobilinogen, ketones (acetoacetic acid), protein, nitrite, glucose, pH, specific gravity, leukocytes and ascorbic acid in urine. This strip can be read visually or with the AnyScan 720 Analyzer, AnyScan 300 Analyzer, and CK-60 Urine Analyzer. No additional reagents or laboratory equipment is required. The reagent strips are packaged in a plastic vial containing desiccant. The test strips must be tightly capped in the plastic vial to assure reagent reactivity. The directions must be followed exactly, and it is necessary to use fresh, well m xed and that has not been centrifuged urine for optimal results.

### CHEMICAL PRINCIPLES OF THE PROCEDURE

**COMPENSATION AREA:** is a white area, which is not mpregnated with reagents to allow compensation for the intrinsic color of the urine while testing for the parameters.

**BLOOD:** This test is based on the pseudoperoxidase activity of hemoglobin which catalyzes the reaction of 3, 3', 5, 5'-Tetramethylbenzidine and buffered organic peroxide, Cumene hydroperoxide. The resulting color ranges from yellowish green through green to dark green.

**BILIRUBIN:** This test is based on the coupling of bilirubin with 2,4 dichlorobenzene diazonium Na in a strong acid medium. The color changes from light tan to purple.

**UROBILINOGEN:** The test is based on the diazotization reaction of 4-Methoxybenzene diazonium salt and urinary urobilinogen in a strong acid medium. The color changes range from pink to brown-red.

**KETONES:** This test is based on the reaction of acetoacetic acid in the urine with nitroprusside. The resulting color ranges from tan, when no reaction takes place, to purple for positive reaction.

**PROTEIN:** The test is based on the color change of the indicator, tetrabromphenol blue, in the presence of protein. A positive reaction is indicated by a color change from yellow through green and then to greenish-blue.

**NITRITE:** This test is based on the reaction of p-arsanilic acid and nitrite (which is derived from dietary nitrate in the presence of bacteria) in urine to form a diazonium compound. The diazonium compound in turn couples with N-(1-naphthyl) ethylenediamine in an acidic medium. The resulting color is pink. Any degree of pink color is considered positive.

**GLUCOSE:** This test is based on a sequential enzyme reaction. First, glucose oxidase catalyzes the formation of gluconic acid and hydrogen peroxide from the oxidation of glucose. A second enzyme, peroxidase, catalyzes the reaction of hydrogen peroxide with potassium iodide chromogen to oxidize the chromogen to colors ranging from blue through greenish-brown and brown to dark-brown.

**pH:** This test is based on double indicators (methyl red and bromothymol blue) which give a broad range of color covering the entire urinary pH range. Colors range from orange through greenish-yellow and green to blue.

**SPECIFIC GRAVITY:** This test is based on the pKa change of certain pretreated polyelectrolytes in relation to ionic concentration. In the presence of an indicator, color ranges from deep blue in urines of low ionic concentration through green and yellow-green in urines of increasing ionic concentration.

**LEUKOCYTES:** This test reveals the presence of granulocytes esterases. The esterases cleave a derivatized thiazole amino acid ester to liberate derivatized hydroxythiazole. This thiazole then reacts with a diazonium salt to produce a purple product.

**ASCORBIC ACID:** This test is based on the reducing process of ascorbic acid. The composition comprises aromatic compounds which are colored in their oxidized state but which become colorless when reduced by ascorbic acid. As urinary ascorbic acid can interfere with tests for blood, bilirubin, nitrite and glucose, an ascorbic acid test pad is included to enable a technician to evaluate the results of urinalysis in light of this potential source of interference.

**Reagent** content is based on dry weight at the time of impregnation of 100 strips:

| | | |
|---|---|---|
| BLOOD | 2, 5-Dimethylhexane-2, 5-dihydroperoxide | 40 mg |
| | 3,3',5,5'-Tetramethylbenzidine | 3.7 mg |
| BILIRUBIN | 2, 4-Dichlorobenzenediazonium salt | 3.0 mg |
| | Oxalic acid | 30.0 mg |
| UROBILINOGEN | 4-Methoxybenzenediazonium salt | 2.5 mg |
| | Citric acid | 30.0 mg |
| KETONES | Sodium nitroprusside | 20.0 mg |
| | Magnesium sulfate | 246.5 mg |
| PROTEIN | Tetrabromphenol blue | |
| NITRITE | p-Arsanilic acid | 5.0 mg |
| | N-(1-naphthyl)ethylenediamine | 6.0 mg |
| GLUCOSE | Glucose oxidase | 451 units |
| | Peroxidase | 186 unit |
| | Potassium iodide | 10.0 mg |
| pH | Methyl red | 0.04 mg |
| | Bromthymol blue | 0.5 mg |
| SPECIFI GRAVITY | Diethylenetriamine pentaacetic acid | 12.0 mg |
| | Bromthymol blue | 1.2 mg |
| LEUKOCYTES | Indoxylcarbonic acid ester | 1.0 mg |
| | Diazonium salt | 0.7 mg |
| ASCORBI ACID | 2, 6-dichlorophenol indophenols | 1.60mg |

### STORAGE

Store at room temperature between 15℃ to 30℃ (59°F to 86°F). Do not store the strips in the refrigerator or freezer. Re-cap the bottle tightly immediately after use. Since the test strips are sensitive to specific environmental factors, such as moisture, heat and light, do not expose strips to these factors.

### PROCEDURE FOR HANDLING THE STRIPS

Al unused strips must be stored in the original bottle. Transfer of the strips to another container may cause reagent strips to deteriorate and become unreactive. Reagent strips should always be stored in their dedicated vial and should be kept tightly capped. After taking out test strips, replace the cap promptly and tightly. Don't touch test area of the strip. Do not use strips after expiration date. The work area should be clean & free of detergents and other contaminants. Protect reagent strips from moisture, heat and light.

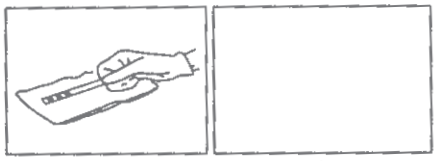
### SPECIMEN COLLECTION

Collect urine in a clean, dry, unused vessel. Test urine as soon as possible after collection. If testing cannot be performed within an hour after voiding, refrigerate the specimen immediately and let it return to room temperature before testing.

### TEST PROCEDURE

This procedure ***MUST BE FOLLOWED EXACTLY*** to achieve reliable test results

1. Confirm that the product is within the expiration date shown on the label.
2. Remove the strip from the bottle and replace the cap immediately.
3. Inspect the strip, Discoloration or darkening of reagent areas may indicate deterioration. Do not use the strip.
4. Dip the test strip completely for no more than 1 second in fresh, well- mixed, and



that has not been centrifuged urine specimen. Excessive urine on the test strip may give rise to a wrong result. Remove the excessive urine by touching the plastic film on the rim of vessel. At this time, do not allow the reagent areas to touch the rim of vessel. Excessive urine may be removed by gently blotting the lengthwise edge on absorbent paper.

5. Compare the test results carefully with the color chart on the bottle label in good light. Proper reading time 60 seconds (120 seconds for the leukocyte test area) is critical for optimal results. While comparing, keep the strip in a horizontal position to avoid possible interaction of chemicals by excessive urine. Changes in color that appear only along the edges of the test areas or after more than two minutes have passed are of no diagnostic significance.

### QUALITY CONTROL

The strips must be properly stored and handled before and during the testing. Reaction of reagent strips should be confirmed by testing known positive and negative specimens or multiple analyte controls containing normal and abnormal amounts of each of the analytes being tested. For quality control, urine controls commercially available can be used.

### RESULTS

The results are obtained by direct comparison of test strip with the color chart printed on the bottle label. No calculations or laboratory instruments are necessary. For visual readings, the compensation pad may be used to determine if highly pigmented urine or colors appear that may confuse the proper interpretation of the analyte colors.

### LIMITATIONS

Substances that cause abnormal urine color, such as drugs containing azo dyes, nitrofurantoin and riboflavin may affect the readability of reagent areas on urinalysis reagent strips.

The color development on the reagent pad may be masked, or a color reaction may be produced on the pad that could be interpreted visually and instrumentally as a false positive. It is therefore recommended that in case of doubt, the test should be repeated after withdrawal of the medication.

**BLOOD:** A false positive reaction can sometimes occur when bacteria are present in urine or if the urine contains residues of strongly oxidizing cleaning agents from the container. Elevated ascorbic acid or proteins have no effect on the test. Strong oxidizing substances, such as hypochlorites, may produce a false positive result. Urine from menstruating females often, but not always, yields positive results.

**BILIRUBIN:** Normally no bilirubin is detectable in urine by even the most sensitive methods. Since the bilirubin in specimens is sensitive to light, prolonged exposure of the urine sample to light can cause oxidation resulting in lower or false negative values. In addition, higher ascorbic acid concentration or the presence of diagnostic or therapeutic dyes in the urine may also cause false negatives results. Even trace amounts of bilirubin are sufficiently abnormal to require further investigation.

**UROBILINOGEN:** A complete absence of urobilinogen in the specimen being tested cannot be demonstrated by the strip. Normal urine specimens ordinarily give a slight pink color. Higher concentrat

**KETONES:** Normal urine specimens ordinarily yield negative results with this reagent. False positive results may occur with highly pigmented urine specimens or those containing large amounts of levodopa metabolites.[1] Whereas, prolonged room temperature storage of urine can result in a false-negative results for ketones. Urine should not be stored at room temperature for more than **two hours.**

**PROTEIN:** The minimum sensitivity of this test is 5 ~ 10 mg/dl of protein in urine. Highly buffered alkaline urines (pH>9), traces of disinfectants or various types of medication may give false positive results.[2] The interpretation of results is also difficult in turbid urine specimens.

**NITRITE:** Any degree of uniform pink color development should be considered positive however, pink spots or pink edges should not be interpreted as a positive result. Color development is not proportional to the number of bacteria present. The nitrite test detects only nitrate reducing bacteria. Occasionally bacteria will be present that do not reduce nitrate to nitrite. Therefore, mid-stream of first morning urine specimen is recommended for this test.[3] Sensitivity of the nitrite test is decreased with high specific gravity or ascorbic acid concentrations of 20 mg/dl or greater. False negative results can occur during antibiotic therapy.

**GLUCOSE:** Reactivity of the test decreases as the specific gravity and/or pH of urine increases, and may also vary with temperature. Ascorbic acid (more than 40 mg/dl) and ketone bodies (more than 50 mg/dl) may cause a false negative for a specimen containing a small amount of glucose (100 mg/dl) however, the combinations of such ketone levels and low glucose levels are metabolically improbable in screening. False positive reactions can be caused by traces of detergents containing peroxide or other interfering ingredients. Vitamin C doses of 250 ~ 500 mg or more may cause false negative results.

**pH:** This pH test indicates the pH values only within the range of 5 to 9. Certain drugs, such as those used for hypertension and heart trouble (Acetazolamides) may cause alkaline urine.[4]

**SPECIFIC GRAVITY:** The test determines the ionic concentrations in urine. Non-ionic elements such as glucose or urea are not analyzed. Therefore it is recommended that the urine density is checked using a refractometer or a hydrometer.

**LEUKOCYTES:** The test result may not always be consistent with the leukocyte cell number by the microscopic examination.[5] False positive results may be found high humidity and high temperature condition, and failure of the bottle security. And also positive results may occasionally be found with the random specimens from females due to contamination of the specimens by the vaginal discharge. High concentration of glucose, specific gravity, protein, and formaldehyde, or presence of blood may reduce the reactivity of test results. Moreover, high concentration of oxalic acid or trace of oxidizing agents may cause false negative results. Low specific gravity (1.010 and under) may cause a false positive.

**ASCORBIC ACID:** False positive reaction may be obtained with other reducing agent.

## EXPECTED VALUES

**BLOOD:** Hemolysis is a natural process of recycling old or damaged red cells. But when hemoglobin appears in urine, it indicates kidney disease or urinary tract disorder. The practical detection limit of this test is approximately 10 erythrocytes per microliter of urine. Blood may be found in the urine of menstruating females. This test is highly sensitive to hemoglobin (it is slightly less so to intact erythrocytes) and thus complements the microscopic examination.

**BILIRUBIN:** No bilirubin is detectable in urine of healthy persons by even the most sensitive methods. Elevated bilirubin in urine always indicates disease and is the earliest sign of liver cell disease and/or biliary obstruction. The signs of "+" (0.5 mg/dl), "++" (1.0 mg/dl), and "+++" (3.0 mg/dl) signify the qualitative severity of the liver damage or bile obstruction. Even trace amounts of bilirubin are sufficiently significant to require further investigation.

**UROBILINOGEN:** In this test strip, the normal urobilinogen range is 0.1 ~ 1.0 mg/dl (1 mg/dl is approximately equal to 1 Ehrlich unit/dl).[3] If results exceed the concentration of 2.0 mg/dl, the patient and/or the urine specimen should be evaluated further.

**KETONES:** Ketone bodies should not be detected in normal urine specimens with this reagent. The concentrations given: "±" (5 mg/dl), "+" (10 mg/dl), "++" (50 mg/dl), "+++" (100 mg/dl) correlate well with the acetoacetic acid concentration in urine. The sensitivity of this test is 5 mg acetoacetic acid per 100 ml of urine. Detectable levels of ketone may occur with frequent vomiting, diarrhea, digestive disturbances, pregnancy, or severe physical exercise.[4]

**PROTEIN:** Normal urine specimens ordinarily contain some protein (0 ~ 4 mg/dl); therefore, only persistent elevated levels of urine protein indicate kidney or urinary tract disease. The persistent results of a trace level or greater indicate significant proteinuria, and thus further clinical testing is needed to evaluate the significance of results. The concentration given: "+" (30 mg/dl), "++" (100 mg/dl), "+++" (300 mg/dl), "++++" (1000 mg/dl) correlate well with the albumin concentrations in urine. Pathologic proteinuria generally gives persistent values over 30 mg/dl.

**NITRITE:** Testing of urine for nitrite is a test for bacteria in urine. Any degree of pink color after 30 seconds indicates clinically significant bacteriuria. Bacteriuria is generally due to infection of the kidneys, ureters, bladder or urethra.

**GLUCOSE:** Normally no glucose is detectable in urine, although a minute quantity of glucose is excreted by the normal kidney. Approximately 50 ~ 100 mg glucose/dl of urine is detectable in this test strip. Concentrations of 100 mg/dl may be considered as abnormal if found consistently.

**pH:** Normal urine is slightly acidic with a pH of 6. Urine pH values generally range from 5 to 8. The pH of urine is an important indicator of certain metabolic, kidney, gastrointestinal and respiratory factors.

**SPECIFIC GRAVITY:** Random urine specimens from adults may vary in specific gravity from 1.001 ~ 1.030. Twenty-four hour urines from normal adults with normal diets and normal fluid intake will have a specific gravity of 1.016 ~ 1.022. This test permits determination of urine SG between 1.000 and 1.030.

**LEUKOCYTES:** Normally no leukocytes are detectable in urine. Individually observed trace results may be of questionable clinical significance.

**ASCORBIC ACID:** High concentration of ascorbic acid in the urine could be found in individuals who routinely ingest doses of vitamin C. It can interfere with urinalysis by strip for glucose, occult blood, bilirubin and nitrite. If ascorbic acid is detected in urine, the test should be done at least after 24 hours of the last dose of vitamin C.

## PERFORMANCE CHARACTERISTICS

Specific performance characteristics of the SELF-STIK products are based both on clinical and laboratory studies. A study done at two clinical sites involving 94 patient samples compared SELF-STIK to a competitor's strip. 100% agreement within one color block was obtained for all analytes except protein. Protein gave a greater than 95% agreement. The lower agreement may be reflective of the technician's interpretation of the negative versus trace color block with both the SELF-STIK and the competitive strip. Parameters of importance to the user are sensitivity, limits of test, specificity, accuracy, precision and stability. Sensitivity and limits of tests are the generally detectable levels of each test described previously. The sensitivity depends upon several factors; the variability of color perception; the presence or absence of inhibitory factors typically found in urine, the specific gravity, ascorbic acid, and pH; and lighting conditions when the product is read visually. The tests have been developed to be specific for the constituent to be measured with the exception of interferences listed previously. Exact agreement between visual results and instrumental results might not be found because of the inherent differences between the perception of the human eye and the optical system of the instruments. It is for this reason that each user is encouraged to develop his own standards for performance. The stability test has been developed by statistical procedure for various environmental conditions.

**BLOOD:** This test is slightly more sensitive to free hemoglobin and myoglobin than to intact erythrocytes. The test is generally capable of detecting 0.015 mg/dl free hemoglobin or 5 ~ 10 intact red blood cells per microliter urine. The sensitivity may be reduced in urines with high specific gravity and ascorbic acid content. The appearance of Green spots on the reagent test area indicates the presence of intact erythrocytes in the urine. The test may be positive because of hematuria, hemoglobinuria or myoglobinuria.

**Bilirubin:** The test has a sensitivity of 0.5 mg/dl bilirubin. Bilirubin in urine indicates liver disease before any clinical signs are usually evident.

**Urobilinogen:** This test can detect urobilinogen in concentrations as low as 0.1 mg/dl (approximately 0.1 EU/dl); therefore, most normal urines may give a slight pink reaction.

**Ketones**: The reaction of this reagent pad is caused by acetoacetic acid in urine, acetone or β-hydroxybutyric acid makes no significant contribution to this test. Ordinarily, the reagent area detects 5.0 mg of acetoacetic acid in 100 ml urine. Some high specific gravity and low pH urines may give reactions up to and including trace level (5.0 mg/dl).

**Protein:** The test is more sensitive to albumins than to gamma-globulins, Bence-Jones proteins, and mucoproteins; such proteins do not interfere with the reaction of albumin.

**Nitrite:** This test has a sensitivity of 0.05 mg/dl nitrite ion or the amount of bacteria about $10^5$/dl in urine of normal specific gravity and moderate levels of ascorbic acid. The test is specific for nitrite and independent of urinary pH of any other substance normally excreted in urine. Comparison of the reacted area against a white background may aid in the detection of low levels on nitrite.

**Glucose:** The test has a sensitivity of 50 ~ 100mg glucose in 100ml urine, and is specific for glucose. No substance excreted in urine other than glucose is known to give a positive result. False negative results may be obtained with the presence of levodopa, ascorbic acid, glutathione, and dipyrone. If the test color appears somewhat mottled at the higher glucose concentrations, match the darkest color to the color blocks.

**pH:** This test produces distinct color changes from orange to blue over the pH value 5 ~ 9. Values will be read to within 1 unit; however, an accurate reading may be confused because of slight variations caused by the pigments in the urine.

**Specific Gravity:** This test permits determination of urine specific gravity of 1.000, 1.005, 1.010, 1.015, 1.020, 1.025 and 1.030. Highly buffered alkaline urines may cause low reading of result.

**Leukocytes:** The test is generally capable of detecting 15 ~ 25 WBC/μl as a trace.

**Ascorbic Acid:** Approximately 5 mg/dl of ascorbic acid is detectable.

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