**ETHYLENE GYCOL (10 Minute Test)
A.K.A. (EG) ANTI-FREEZE POISON TEST**

**SUMMARY & INTENDED USE**
The KACEY® Anti-Freeze EG/OX COMBO Test Strips are intended to provide a quantitative measurement of Ethylene glycol in “PLASMA”. The KACEY® Anti-Freeze Combo Test Strips measurements are used in the diagnosis and treatment of Ethylene Glycol Poisoning (A.K.A. ANTI-FREEZE POISON). Ethylene glycol is a toxic alcohol that can be found in many commonly used household and automotive products such as ANTI-FREEZE, DETERGENTS, DEICERS and LACQUERS. Ethylene glycol is odorless, colorless and has a relative sweet like taste. It is absorbed very rapidly from the gastrointestinal tract and can reach peak serum concentration levels within one (1) hour after ingested. STAGE 1 Hepatic metabolism of the Ethylene glycol converts the parent compound to Oxalate in a series of steps. Alcohol dehydrogenase converts most of the Ethylene glycol to Glycoaldehyde. This first step is rate limiting and can be blocked through the competitive inhibition of alcohol-dehydrogenase with an agent such as Ethanol or the Kacey Antidote 4MP (Fomepizole). STAGE 2 Glycoaldehyde is metabolized to glycolic acid which in turn is then converted to glyoxylic acid and finally to Oxalate. Unmetabolized Ethylene glycol (ANTI-FREEZE) and its metabolites are eliminated renally.

The KACEY® Anti-Freeze EG/OX Combo Test Strips measures the presence of both Ethylene glycol and Oxalate in a PLASMA sample. A drop of plasma (10uL) is placed onto each of the two test pads on the test strip. After waiting the required 10 minutes the color that forms in the test pad on the strip is compared to a color chart to determine both the presence and concentration of the Ethylene glycol and Oxalate in mg/dl. The Level of Detection (LOD) starts at 10 mg / dl and is Excellent in determining Ethylene Glycol Poisoning in both CATS & DOGS.

**CHEMICAL COMPOSITION**
KACEY® Anti-Freeze Test Strips do not contain any harmful chemicals and is non-hazardous and non caustic.

**STORAGE & HANDLING (Room Temperature)**
- Store the EG/OX Combo Vial in the refrigerator.
- Keep away from heat & direct sunlight.
- Always replace vial caps immediately after removing a test strip from the bottle.

A PLASMA SAMPLE IS REQUIRED – DO NOT USE SERUM OR HEMOLYZED SERUM FOR THIS TEST

**PROCEDURE**
1) PLACE A MINIMUM 300 – 750 µL OF A WHOLE BLOOD SAMPLE INTO A GREEN TOP LITHIUM TUBE. PLACE THE TUBE IN A CENTRIFUGE AND SPIN THE TUBE AT BLOOD SPEED FOR 3-5 MINUTES TO SEPARATE PLASMA FROM THE RED BLOOD CELLS.
2) REMOVE THE EG/OX COMBO TEST VIAL FROM THE REFRIGERATOR, AND REMOVE ONE TEST STRIP FROM THE VIAL IMMEDIATELY RECAP THE VIAL.
3) PLACE ONE DROP OF PLASMA (10uL) FROM THE ABOVE CENTRIFUGE D TUBE (APPROXIMATELY 10 µL) ONTO EACH OF THE TWO PADS ON THE TEST STRIP. THE 10UL PIPETTORS ARE AVAILABLE FROM KACEY®
4) WAIT FOR 10 MINUTES AND COMPARE THE COLOR OF EACH OF THE TWO (2) PADS TO THE COLOR CHART BLOCK ON THE VIAL. DO NOT READ THE COLOR STRIP AFTER 10 MINUTES AS THE COLOR WILL START TO DETERIORATE AND YIELD ERRONOUS RESULTS

**PRINCIPLE OF THE TEST**
If Ethylene glycol is present a BLUE GREEN (TEAL) COLOR will appear on the “EG” pad. If Oxalate is present a BLUISH color will appear on the oxalate test pad. SEE DIFFERENT VALUES ON THE SECOND PAGE OF BOTH THE EG & OXALATE INSERT. HERE SYOU WILL SEE THE THE DIFFERENT RESULTS FOR BOTH PADS. MATCH THAT COLOR OF THE TEST PAD TO THE COLOR BLOCK CHART ON THE VIAL. IF NO DISTINCT BLUE GREEN COLOR DOT APPEARS THROUGHOUT THE ENTIRE PAD THE TEST IS NEGATIVE (SEE TEST RESULT SECTION)

The intensity of the color is directly proportional to the concentration of the ethylene glycol. Propylene glycol, glycerol, ethanol, medication products containing alcohol, and synthetic sugars for baking (Ex. Splenda, manitobol) will give false positive results.

**NOTE: DO TO THE PHENOMENA OF WICKING DARKER COLORS MAY APPEAR AROUND THE EDGES OF THE TEST PAD**

**SPECIMEN COLLECTION AND PREPARATION**
Collect enough whole blood (with GREEN TOP TUBE LITHIUM HEPARIN Anticoagulant) to perform the test. Blood can be collected from the animal by a direct blood draw using a needle.

Plasma can be obtained by centrifugation to separate the red blood cells from the plasma.

THE TEST MUST BE PERFORMED ONLY ON A PLASMA SAMPLE

**QUALITY CONTROL**
Good laboratory Procedures for QC (Quality Control) should be followed: EX. ALL Pos. (+) & Neg (-) results should be confirmed by running both Positive & Negative Controls for verification that the test result is indeed positive or negative. KACEY® provides Ethylene Glycol Controls: 0, 10 and 50 mg/DL. These Optional Controls are available separately and can be ordered directly from Kacey®. The user can practice the testing with these controls to get familiar with the test procedure and colors for negative and positive results. Kacey highly recommends that the tester should always verify all positive / negative results of the patient by comparing the color results with that of the Bi-Level control as a 2nd source of validating the (+) &(-) test results.

**PRECAUTIONS**
*For in vitro diagnostic use only.
*Out of date or expired strips should never be used to perform a test. Check the vial for the expiration date.
*If the sample pad is covered with Plasma, sufficient sample has been applied.
*If an insufficient amount of Plasma is placed on the strip, do not add Plasma to the same strip. Use a new unused test strip.
*Do not agitate the strip after the plasma sample is added.

**TEST RESULTS**
Results are displayed in “milligrams per deciliter” of ethylene glycol per milligrams per deciliter (mg/dl) and can be converted to (μg/ml). Therefore, a 20 mg / dl is equal to 200 μg / ml.

A FRAGMENTED BROWNISH-YELLOWISH COLOR OR ANY FRAGMENTED PARTS IN THE PAD IS CONSIDERED NEGATIVE.

**LIMITATION OF THE PROCEDURE**
- Ethanol interferes with the test, and will give false positive ethylene glycol test results.
- Toxibian will interfere and cause False Positives unless test is performed before Toxibian is administered.
- All alcohol or compounds that can be converted into alcohol within the body will results in False positives. This also can include but NOT limited to artificial sugars used in baking or cooking.
EXPECTED VALUES - LOD = Level of Detection LD=LETHAL DOSAGE LD FOR A CAT IS 20 mg/dL LD FOR A DOG IS 50 mg/dL

Measuring range: The Kacey® antifreeze test can measure ethylene glycol values starting from 10 mg/dL which is equivalent to 100 µg/ml and above. This LOD is low enough to detect Ethylene Glycol Poisoning in Cats and is excellent in early detection of Ethylene Glycol Poisoning in small, medium and large size dogs. The color chart enclosed provides four (4) color blocks in order to measure the ethylene glycol results. Negative, 20 mg/dL, 50 mg/dL, and 75 mg/dL. Cats showing a color greater then or equal to the 10mg/dL color block is positive, dogs showing a result in which the color is greater than or equal to the 10 mg/dL color block would be considered positive. (Note: it is possible for a dog to have a value greater than 10 mg/dL and less than 50 mg/dL. This would suggest that ethylene glycol had been ingested and should be viewed as an ethylene glycol poisoning even if it is not considered to be at a lethal dosage level above the 50 mg/dL threshold mark.

NOTE: IT IS RECOMMENDED AS A GOOD LABORATORY TESTING PROCEDURE TO RUN CONTROLS ON THIS ETHYLENE GLYCOL TEST TO CONFIRM AND OR VALIDATE ALL POSITIVE AND NEGATIVE RESULTS. THE KACEY COMPANY PROVIDES BI-LEVEL CONTROLS WHICH CAN BE PURCHASED TO VALIDATE THE RESULTS OF THE EG TEST. TO ORDER BI-LEVEL CONTROLS PART # 30304 EMAIL YOUR ORDER TO: sales@kaceyinc.org

Note: It is “Estimated” that the amount of ½ tsp of anti-freeze (Conc. 95%+) per 1 pound of canine would be a lethal dosage of antifreeze for the average healthy canine. It has been reported that the current recommended standard for a lethal dose of antifreeze (Conc. 95%+) is at a threshold level of 50 mg/dL (Equivalent to 500 µg/ml) and above. The size, weight, age, metabolism, and health condition of the animal plays a major role on the assimilation and toxic response to the amount anti-freeze ingested.

Estimated detection @10mg/dL is approximately 30-45 minutes after ingestion with a window of opportunity up to 9-10 hours: See Note above In the section for Expected values

ACCURACY

The ANTIFREEZE POISON TEST STRIPS were used against a commercially available test kit, Ethylene Glycol Test Kit by Allelic Biosystems, confirmed by spiked PLASMA samples with known ethylene glycol concentrations.

THE KACEY® ETHYLENE GLYCOL (ANTIFREEZE) POISON TEST STRIPS COMPARES WELL TO THE REFERENCE METHOD.

RE-ORDER PART# 30301 (FIVE TESTS PER KIT) 30325 -10 µL PIPETTOR 30328 (50 -PIPET TIPS)

TEST RESULT POSSIBILITIES FOR PLASMA ETHYLENE GLYCOL AND PLASMA OXALATE: Shown is the new combination EG and OX on one test strip which accurately tracks the progression of Ethylene Glycol through the two stages after exposure. As you can see in end Stage 2 Ethylene Glycol detection drops to zero and Oxalate levels signifying a poor prognosis necessitating the use of Oxalate detection.

DO NOT USE THESE COLOR CODES. USE ONLY THOSE SUPPLIED ON VIAL LABEL. THESE ARE FOR REFERENCE PURPOSES ONLY.
Oxalate is a metabolic breakdown product of the Krebs’s Cycle in eukaryotes, and the glyoxylate cycle in other microorganisms. It can be found in the urine of humans and other mammals. Oxalate concentration can be used as a measure of kidney function in chronic kidney disease where a high level of oxalate is an indicator for kidney stones which are primarily made of the insoluble salt calcium oxalate. Ingested Ethylene Glycol is rapidly absorbed and metabolized in the liver over 8-10 hours to glycolic acid which is eventually converted to oxalic acid. Oxalic acid then combines with calcium to form insoluble calcium oxalate crystals which damage the kidneys. Measuring plasma oxalate is more accurate than measuring plasma calcium as a marker for kidney stones because calcium is excreted at high concentrations even in normal urine. Kaceys Diagnostics’ Oxalate Test Strips combine the oxalate oxidation reaction and color reaction in one step on a plasma sample. The intensity of product color is directly proportional to the oxalate concentration in the sample.

The KACEY® Anti-Freeze EG / Oxalate Combo Test Strips are intended to provide a quantitative measurement of Oxalate in “PLASMA” 8-10 hours post ingestion. The KACEY® Anti-Freeze Oxalate Test Strip measurements are used in the diagnosis and treatment of Ethylene Glycol Poisoning (A.K.A. ANTI-FREEZE POISON ). Ethylene glycol is a toxic alcohol that can be found in many commonly used household and automotive products such as ANTI-FREEZE, DETERGENTS, DEICERS and LACQUERS. Ethylene glycol is odorless, colorless and has a relatively sweet like taste, but even when a bitterant is added it seems to attract animals who invariably ingest it. It is absorbed very rapidly from the gastrointestinal tract and can reach peak serum concentration levels within hours of ingestion. Hepatic metabolism of the Ethylene glycol converts the parent compound, Glycolic acid to Oxalate in a series of steps. During this process there is a window of opportunity from -0.8-10 hours in which the Kacey Ethylene Glycol test (EGT) strips can determine exposure. However, after this time depending on weight, species and amount of ingestion, Ethylene Glycol converts to an Oxalate and the Kacey EGT strips may indicate a false negative on exposure. The Kacey Oxalate test will effectively measure the presence of oxalate in the secondary phase when the glycolic acid has been converted to oxalate. Knowing if there is oxalate present or not with a positive Ethylene Glycol test allows for the correct detoxification modality to be employed. (SEE CHART BELOW) Often pets are not presented until many hours post ingestion and this second phase test from Kacey becomes critical. The detox modality for each of these two phases is different and therefore it is critical that if possible both tests be administered concurrently to determine exposure time. Alcohol dehydrogenase converts most of the Ethylene glycol to Glycol aldehyde. This first step is rate limiting and can be blocked through the competitive inhibition of alcohol-dehydrogenase with an agent the Kacey Antidot 4MP (Forniphizes) however, after the 8-10 hour mark, Glycol aldehyde is metabolized to glycolic acid which in turn is converted to glyoxylic acid and finally to an Oxalate. This second phase (stage 2) when combined with calcium precipitates Calcium Oxalate crystals which precipitate in the kidneys leading to severe renal tubular damage and potential acute renal failure.

TEST RESULT POSSIBILITIES FOR PLASMA ETHYLENE GLYCOL TEST (EGT) AND PLASMA OXALATE TEST

EGT NEGATIVE OXALATE NEGATIVE NO EXPOSURE

EGT POSITIVE OXALATE NEGATIVE EARLY EXPOSURE 4MP

EGT POSITIVE OXALATE POSITIVE LATER EXPOSURE 4MP and IV fluids

EGT NEGATIVE OXALATE POSITIVE POOR PROGNOSIS IV Fluids (4MP ineffective at this stage)

Ethylene glycol toxicity causes acute renal failure! Fluid diuresis and correction of acidosis are important parts of therapy. Prognosis is good with early aggressive treatment (<8 hours of ingestion) with 4MP, but poor or grave with prolonged untreated exposure. The total volume of intravenous fluids needed can be calculated using the equation: Maintenance (40 - 60 ml/kg/day) plus Deficit in L (% dehydration * kg body weight) plus insensible losses from vomiting and/or diarrhea. Deficit amounts can be infused rapidly, but so that fluids have time to equilibrate between the vascular, interstitial, and cellular compartments, maintenance fluids must be divided and administered over 8-12 hours. A balanced electrolyte solution can be used (LRS, Normosol®); however, if hyperkalemia exists, 0.9% saline should be substituted. Due to the reduced ability of the kidneys to regulate fluids, Ethylene Glycol renal failure patients are at a high risk for overload and death. If oliguric renal failure occurs, Furosemide, a loop diuretic, has experimentally been shown to increase urine production, dissolve tubular obstructions, and induce vasoconstriction. Often it is given as a bolus (2-4 mg/kg), and if urine production increases, a CRI is started (0.25-1.0 mg/kg/h). An indwelling urinary catheter should be aseptically placed to measure urine output. Hourly urine output is then used to determine "maintenance" fluid rate. Metabolic Acidosis occurs about 12 hours after EG ingestion and lasts up to 24 hours. Severe metabolic acidosis leads to compensatory respiratory alkalosis. Laboratory abnormalities include increased anion gap, decreased serum bicarbonate, and hyperglycemia. Generally, it is not treated unless serum bicarbonate is <14 mEq/L or pH <7.2. A conservative bicarbonate dose can be calculated: (bodyweight in kg x 0.3) x (16 - measured bicarbonate in mEq/L). One-third this dose is given over 30 minutes while another one-third of the dose can be given with intravenous fluids every 4-6 h.

The KACEY® Oxalate test in the Anti-Freeze Combo Test Strips measures the presence of Oxalate in a PLASMA sample after the 8-10 hour mark depending on species, weight and quantity of ingested material. A drop of plasma is placed on the strip pad. After waiting the required 10 minutes the color that forms on the pad of the strip is compared to a color chart to determine both the presence and concentration of an Oxalate in mg/dL Level of Detection (LOD) starts at 0.25 mg / dl (see Expected Value Section Below) Kacey® Oxalate test is Excellent in determining both exposure to Ethylene Glycol Poisoning and respective change to an Oxalate in both CATS & DOGS.

CHEMICAL COMPOSITION
KACEY® Oxalate Anti-Freeze Test Strips do not contain any harmful chemicals and is non-hazardous and non-caustic.

STORAGE & HANDLING (must be refrigerated at 4 C or 39.2°F)
- Store in the Refrigerated ( Do Not Freeze)
- Keep away from heat & direct sunlight
- Always replace vial caps immediately after removing a test strip from the bottle.

A PLASMA SAMPLE IS REQUIRED – DO NOT USE SERUM OR HEMOLYZED SERUM FOR THIS TEST

PRINCIPLE OF THE TEST
If Oxalate is present a LIGHT GREEN TO TEAL COLOR will appear on the pad of the test strip. Match that color in the test strip to one of the color blocks on the color chart on the bottle. If NO DISTINCT LIGHT GREEN TO TEAL COLOR appears THROUGHOUT THE ENTIRE PAD, the test is negative (FRAGMENTS -SEE TEST RESULT SECTION). The intensity of the color is directly proportional to the concentration of the Oxalate.

SPECIMEN COLLECTION AND PREPARATION
Collect enough whole blood (with GREEN TOP TUBE LITHIUM HEPARIN Anticoagulant) to perform the test. Blood can be collected from the animal by a direct blood draw using a needle.

Plasma can be obtained by centrifugation to separate the red blood cells from the plasma.

PROCEDURE
- PLACE A MINIMUM 300 – 750 µL OF A WHOLE BLOOD SAMPLE INTO A GREEN TOP TUBE LITHIUM HEPARIN TUBE
- PLACE THE HEAPARIN TUBE IN A CENTRIFUGE AND SPIN THE TUBE AT BLOOD SPEED FOR 3-5 MINUTES TO SEPARATE PLASMA FROM THE RED BLOOD CELLS.
- PLACE THE COMBO TEST STRIP (EGO&OX) TEST STRIP ON A FLAT SURFACE OR PAPER TOWEL.
- INNOCULATE WITH ONE DROP OF PLASMA FROM THE ABOVE CENTRIFUGED TUBE USING THE KACEY 10µL PIPETTOR (PART #0328) ONTO EACH OF THE TWO (2) TEST PADS ON THE TEST STRIP. ENCOMPASS THE ENTIRE SQUARE PAD WITH THE PLASMA. THE 10µL PIPETTOR AND PIPET TIPS ARE AVAILABLE FROM KACEY®
- WAIT FOR 10 MINUTES AND COMPARE THE COLOR ON EACH OF THE TWO PADS OF THE TEST STRIP TO THE COLOR CHART PROVIDED ON THE BOTTLE. (see illus- trations on back page) DO NOT READ THE COLOR STRIP AFTER 10 MINUTES AS THE COLOR WILL START TO DETERIORATE AND YIELD ERRONIOUS RESULTS.

SUMMARY & INTENDED USE
KACEY® (Stage-2) ANTI-FREEZE POISON TEST
10 MINUTE OXALATE (10 Minute Test)

1545 A Hendersonville Rd
Asheville, NC 28803
Ph: 1(828) 685-3569
Fax: 1(828) 685-3571
www.KaceyDiagnostics.com

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**EXPECTED VALUES**  
**LETHAL OXALATE VALUE FOR A CAT IS 0.25 mg/dL AND 0.25 mg/dL VALUE FOR A DOG**

Measuring range: The Kacey® antifreeze Combo test can measure OXALATE 0.25 mg/dL which is equivalent to 2.5 μg/mL and above. This LOD is also low enough to detect Ethylene Glycol Poisoning in Cats and is excellent in determining post 8-10 hour poisoning of Ethylene Glycol in small, medium, and large size dogs. The color chart enclosed provides five (5) color blocks in order to measure the OXALATE glycol results. Negative (0), 0.25 mg/dL, 0.5 mg/dL, 1.0 mg/dL, and 1.5 mg/dL. Cats & DOGS showing a color greater than or equal to the 0.25 mg/dL color block is positive for exposure to be greater than 8-10 hours.

**QUALITY CONTROL**

Good laboratory procedures for QC (Quality Control) should be followed: EX. ALL Pos. (+) & Neg (-) results should be confirmed by running both positive & negative controls for verification that the test result is indeed positive or negative. KACEY® provides Oxalate Controls: 0, 20 and 80 μg/mL. These Optional Controls are available separately and can be ordered directly from Kacey®. The user can practice the testing with these controls to get familiar with the test procedure and colors for negative and positive results. Kacey highly recommends that the tester should always verify all positive/negative results of the patient by comparing the color results with that of the Bi-Level control as a 2nd source of validating the (+) & (-) test results.

**PRECAUTIONS**

* For in vitro diagnostic use only.

* Out of date or expired strips should never be used to perform a test. Check the vial for the expiration date.

* If the sample circle is covered with Plasma, sufficient sample has been applied.

* If an insufficient amount of Plasma is placed on the strip, do not add Plasma to the same use. New unused test strip.

* Do not agitate the strip after the plasma sample is added.

**LIMITATION OF THE PROCEDURE**

Propylene glycol, glycerol, ethanol, medication products containing alcohol, and synthetic sugars for baking (Ex. Splenda, mannitol) will give false positive results. All alcohol or compounds that can be converted into alcohol within the body will result in False positives. This also can include but NOT limited to artificial sugars used in baking or cooking. Toxiban® will interfere and cause false positives unless test is performed before Toxiban is administered.

Note: It is “Estimated” that the amount of ½ tsp of anti-freeze (conc. 95% +) per 1 pound of canine would be a lethal dosage of antifreeze for the average healthy canine. It has been reported that the current recommended standard for a lethal dose of antifreeze (conc. 95% +) is at a threshold level of 50 mg/dL (Equivalent to 500 μg/mL) and above. The size, weight, age, metabolism, and health condition of the animal plays a major role on the assimilation and toxic response to the amount anti-freeze ingested. Estimated LEVEL OF Detection (LOD) of Oxalate 0.25 mg/dL. Or 2.5 mg/μL is approximately 8-10 hrs after ingestion: See Note above

**ACCURACY**

The ANTIFREEZE POISON TEST STRIPS were used against a commercially available test kit, Ethylene Glycol Test Kit by Allelic Biosystems, confirmed by spiked PLASMA samples with known ethylene glycol concentrations. The Kacey® Ethylene Glycol Poison Test for Oxalate compares well to the reference method.

**RE-ORDER PART**

30310 EG / OX Combo Kit FIVE TESTS PER BOTTLE
30324 10 μL Pipet
30328, PIPET TIPS (50 count)

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**TEST RESULTS**

Results are displayed in “milligrams per deciliter” of ethylene glycol per milliliter (mg/dl) and can be converted to (μg/ml). Therefore, a 20 mg/dl is equal to 200 μg/ml. A FRAGMENTED BROWNISH-YELLOWISH COLOR CONSIDERED NEGATIVE. A DARKER COLOR GREEN MAY APPEAR AROUND THE OUTER EDGES BUT THIS IS ATTRIBUTED TO WHAT WE CALL THE PHENOMENA OF A WICKING EFFECT WHERE A LIQUID WILL TRY TO SEEP TO THE OUTER EDGES OF THE PAD. A TRUE TOTAL BLUE/GREEN COLOR MUST ENCOMPASS THE ENTIRE SQUARE FOR A POSITIVE RESULT.