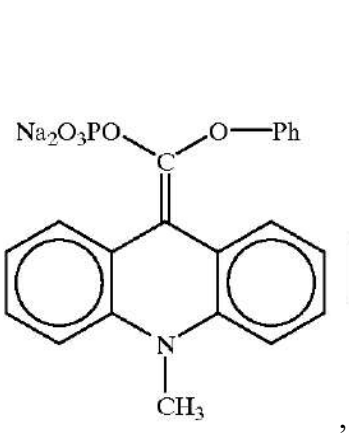
d) 0.05 - 20 g/L of an ether-linked non-ionic surfactant that does not contain a sulfate, sulfonate, phosphate, or carboxylate ester group; or a hydrophilic polymer,
wherein the ether-linked non-ionic surfactant is selected from the group consisting of a polyoxyethylene glycol alkyl ether (BRIJ), a polyoxyethylene glycol octylphenol ether (TRITON), or a polyoxyethylene nonylphenyl ether (IGEPAL).

In some examples, the substrate formulation comprises compound I wherein Z is S; Ar is phenyl or substituted phenyl, wherein substituted phenyl comprises from 1 to 3 halo, C_{1-6} alkyl, C_{1-6} alkoxy, and C_{1-6} haloalkyl substituents; R_1 is methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl or t-butyl; R_7-R_{14} are independently H, C_{1-6} alkoxy, halo; each M is Na or H; and n is 0, 1, or 2.

In specific examples, the substrate formulation comprises a compound I selected from the group consisting of



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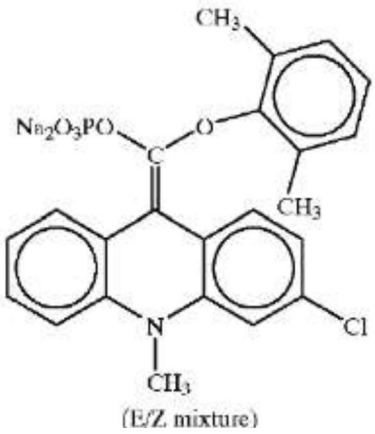


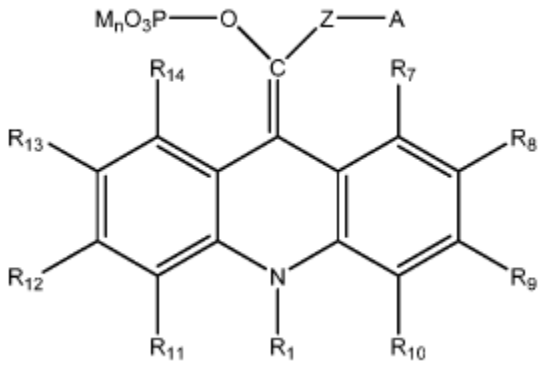
(E/Z mixture) ,

CLAIMS

We claim:

1. A substrate formulation which produces chemiluminescence in the presence of a phosphatase enzyme and which comprises in an aqueous solution:

a) 0.01 mM-50 mM of a chemiluminescent compound of formula I or a salt thereof:



(I)

wherein

A is C_{1-6}haloalkyl, naphthyl, phenyl, substituted phenyl, or heteroaryl, wherein substituted phenyl comprises from 1 to 3 halo, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, $C(O)R_{15}$, CN or NO_2 substituents;

R_1 is selected from the group consisting of C_{5-14}aryl, C_{1-6} alkyl, C_{1-6} haloalkyl, and C_{5-14} aralkyl groups;

R_7-R_{14} are independently H, C_{1-6} alkoxy, halo, or C_{1-4}alkyl, or R_7 -R_8 or R_8 -R_9 or R_9-R_{10} or R_{11}-R_{12} or R_{12}-R_{13} or R_{13}-R_{14}, are joined together as a carbocyclic or heterocyclic ring system comprising at least one 5 or 6-membered ring;

R_{15} is C_{1-6} alkyl;

each M is independently selected from the group consisting of H, an alkali metal, alkaline earth metal, transition metal, ammonium, phosphonium, organic amine salt, and an amino acid salt;

Z is O or S; and

n is 1 or 2;

b) 0.01-200 µM of a cationic aromatic compound (CAC);

c) 1 µM -10 mM of a background reducing agent; and

d) 0.05 - 20 g/L of an ether-linked non-ionic surfactant that does not contain a sulfate, sulfonate, phosphate, or carboxylate ester group; or a hydrophilic polymer,
wherein the ether-linked non-ionic surfactant is selected from the group consisting of a polyoxyethylene glycol alkyl ether (BRIJ), a polyoxyethylene glycol octylphenol ether (TRITON), or a polyoxyethylene nonylphenyl ether (IGEPAL).

2. The substrate formulation according to claim 1, wherein

Z is S;

A is selected from the group consisting of phenyl and substituted phenyl, wherein substituted phenyl comprises from 1 to 3 halo, C_{1-6} alkyl, C_{1-6} alkoxy, or C_{1-6} haloalkyl substituents;

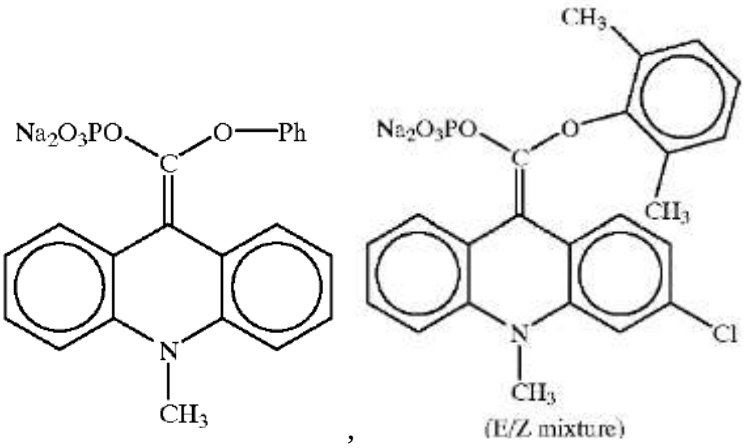
R_1 is methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl or t-butyl;

R_7-R_{14} are independently H, C_{1-6} alkoxy, or halo;

each M is independently H, Na, K, or Li; and

n is 1, or 2.

3. The substrate formulation according to claim 1, wherein the compound of formula I is selected from the group consisting of



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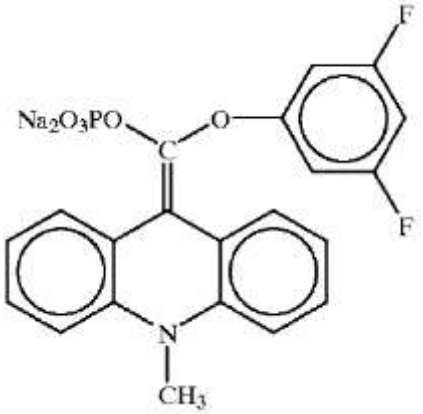

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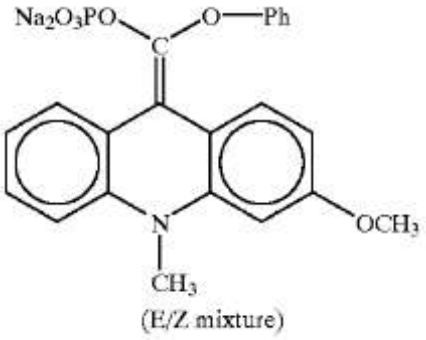
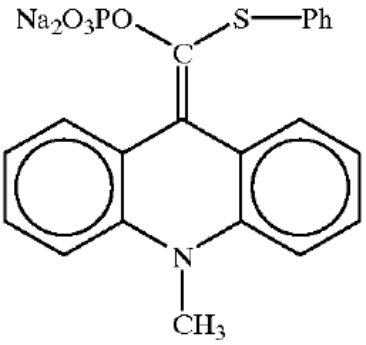


(E/Z mixture)
,



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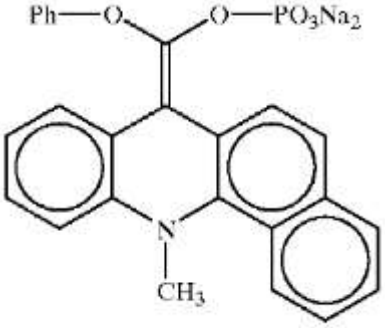

(E/Z mixture)
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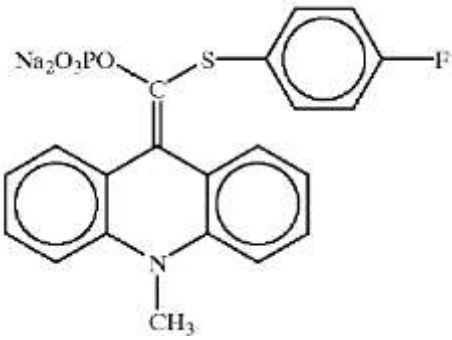

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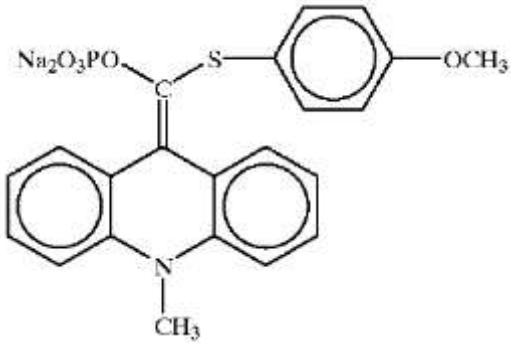

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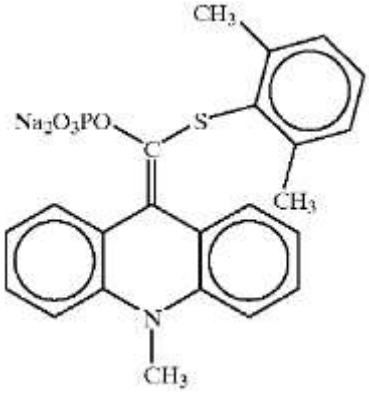

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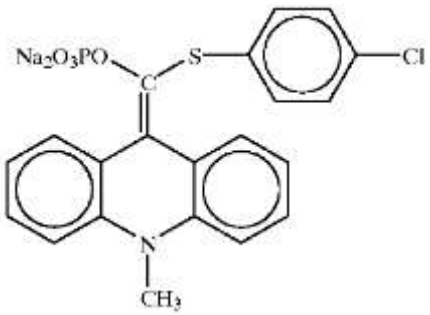

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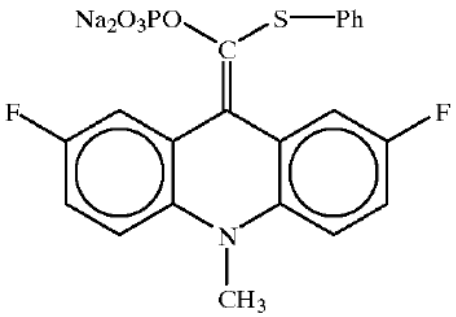
Na2O3PO
S
Ph
C
F
F
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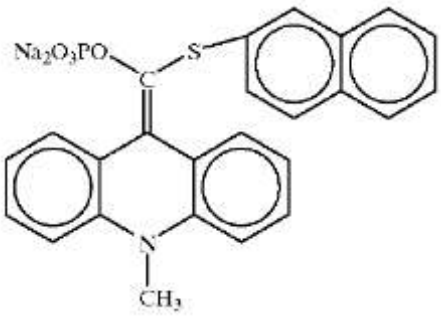
CH3
,

Na2O3PO
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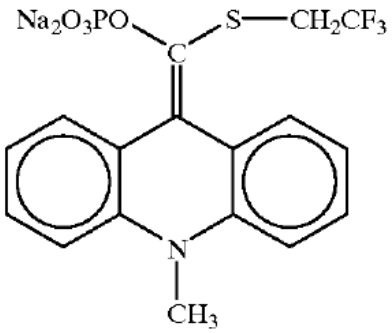
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S
CH2CF3
C
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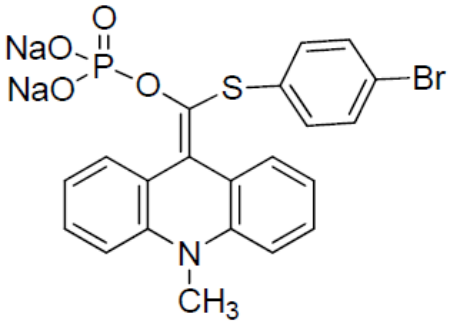
CH3
,

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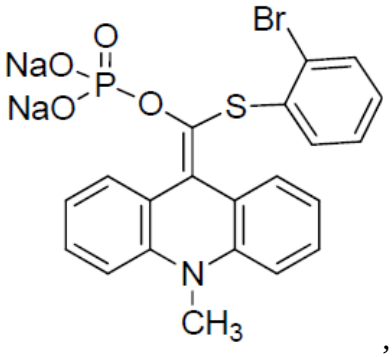
Br
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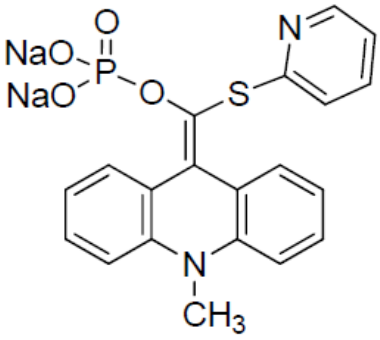
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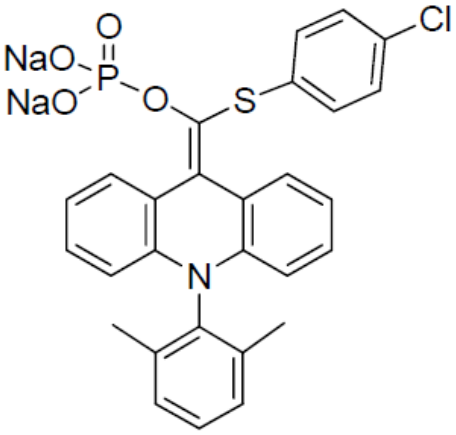
Cl
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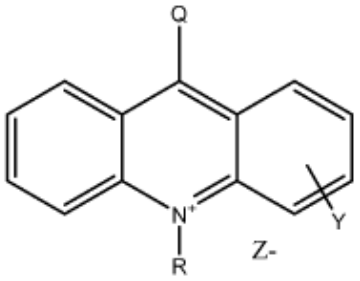
4. The substrate formulation according to claim 1, wherein the cationic aromatic compound (CAC) is selected from a compound according to formula II:



(II)

wherein

Q is selected from the group consisting of halo, cyano, -COOR', -COSR', -$CONR^1$ R^2, -$CON(R)SO_2$ R", naphthyl, anthryl, N-C_{1-4} alkyl acridinyl, and halo substituted N-C_{1-4} alkyl acridinyl;

R is C_{1-4} alkyl;

R', R" are independently selected from the group consisting of C_{1-6} alkyl, aryl, and alkyl substituted aryl;

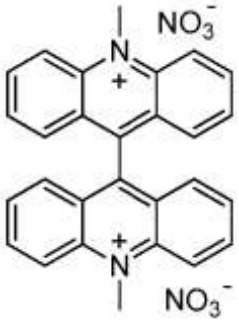
R^1, R^2 are independently selected from the group consisting of H, C_{1-6} alkyl, aryl, and alkylaryl; and

Z^- is a halide or nitrate; and

Y is selected from the group consisting of H, halo, and C_{1-4} alkyl.

5. The substrate formulation according to claim 4, wherein Q is N-methyl acridinyl, halo substituted N-methyl acridinyl, naphthyl, or anthryl; R is C_{1-4} alkyl; Z^- is selected from the group consisting of Cl^-, Br^-, I^-, and NO_3^-, and Y is H or halo.

6. The substrate formulation according to claim 5, wherein the CAC is selected from the group consisting of



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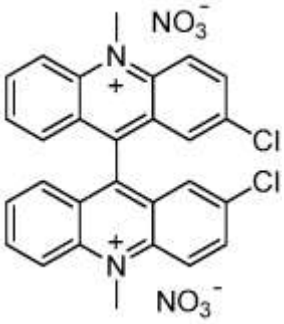

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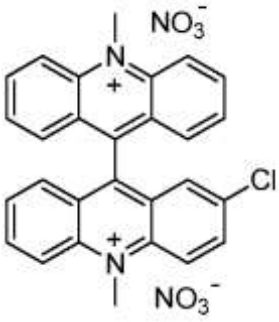

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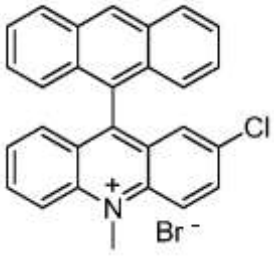

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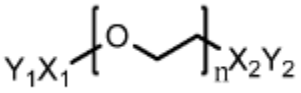
7. The substrate formulation of claim 1, wherein the background reducing agent is selected from the group consisting of lithium sulfite, sodium sulfite, potassium sulfite, lithium bisulfite, sodium bisulfite, potassium bisulfite, lithium metabisulfite, sodium metabisulfite, potassium metabisulfite, dibutylhydroxytoluene (BHT; 2,6-bis(1,10dimethylethyl)-4-methylphenol), butylated hydroxyl anisole (BHA), 3-t-butyl-4-hydroxyanisole, 3-tert-butyl-4-hydroxyanisole, and an aromatic boronic acid of formula Ar-B$(OH)_2$, wherein Ar is phenyl, substituted phenyl, a fused aromatic ring system that may include heteroatom(s), a substituted fused aromatic ring system that may include heteroatom(s), wherein the substituted phenyl or substituted fused aromatic ring system may have from 1-3 substituents independently selected from C_{1-6} alkyl, halo, alkoxycarbonyl, or hydroxyl groups.

8. The substrate formulation of claim 7, wherein the background reducing agent is selected from the group consisting of phenyl boronic acid, 4-tolyl boronic acid, 4-chloroboronic acid, 4-iodoboronic acid and 3-methoxycarbonylphenyl boronic acid, and sodium sulfite.

9. The substrate formulation of claim 1, wherein the hydrophilic polymer is according to formula (IV):



(IV)

wherein X_1 and X_2 are independently selected from O, S, N or NH, or are absent; Y_1 and Y_2 are independently selected from H, H_2 or C_{1-4} alkyl; and n is a number from 20 to 12,000.

10. The substrate formulation of claim 9, wherein the hydrophilic polymer is a poly(ethylene glycol) having an average Mw within the range of 1,000 to 511,000.

11. The substrate formulation of claim 1, further comprising an anionic surfactant selected from the group consisting of C_{10-22}alkyl sulfate and C_{10-22}alkyl sulfonate.

12. The substrate formulation of claim 11, wherein the anionic surfactant is selected from the group consisting of sodium tetradecyl sulfate, sodium dodecyl sulfate (SDS), and sodium tridecyl sulfate (STS).

13. The substrate formulation of claim 1, further comprising an amine buffer selected from the group consisting of Tris (tromethamine); AMPD(2-amino-2-methyl-1,3-propanediol); DEA (diethanolamine); AMPSO(N-(1,1-Dimethyl-2-hydroxyethyl)-3-amino-2-hydroxypropanesulfonic acid); 221-Amine (2-amino-2-methylpropan-1-ol); CHES (2-(N-cyclohexylamino)ethanesulfonic acid); glycine; and TAPS (N-Tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid) buffer at pH 7-12.

14. The substrate formulation of claim 1, comprising 0.05 mM-10 mM compound of formula I, 0.05 - 50 µM cationic aromatic compound, 10 -1000 µM background reducing agent that does not contain a carboxylate ester group, 0.1 to 10 g/L ether-linked non-ionic surfactant or hydrophilic polymer, 0.1 to 5 g/L anionic surfactant, and an amine buffer at pH 8-11.

15. The substrate formulation of claim 14, wherein the compound of formula I is CPA; the CAC is lucigenin; the background reducing agent is sodium sulfite; the non-ionic surfactant is

selected from IGEPAL (CO-990, DM-970), TRITON (X-405, X-405 reduced), and BRIJ (78, 700); the hydrophilic polymer is selected from poly(ethylene glycol) (Mw=14,000), poly(ethylene glycol) (Mw=35,000), and poly(ethylene glycol) (Mw=100,000); the anionic surfactant is selected from SDS and STS; and the amine buffer is selected from TRIS and AMPD.

16. The substrate formulation of claim 1, wherein the substrate formulation exhibits one or more of:

a) achieves maximum intensity (Imax) in < 5 minutes, <4 minutes, <2 minutes, <1 minute, < 45 seconds, < 30 seconds, < 20 seconds, < 10 seconds, < 5 seconds, < 4 seconds, < 3 seconds, or < 2 seconds after exposure to an alkaline phosphatase enzyme;

b) exhibits < 10% loss of original RLU after exposure to a alkaline phosphatase enzyme after storage at 4 ºC for 300 days;

c) exhibits > 90%, or > 95% retained activity (RLU), compared to original RLU, when stored at 4 ºC for 300 days or more;

d) exhibits > 90% retained activity when stored at 4 ºC for 400 days or more; and/or

e) exhibits a signal change in (%) per day after 15 days when stored at 30 ºC of < -0.50 %/day.