

## APPENDIX A

### CHEMICAL REAGENT

#### **0.025 M Potassium chloride buffer (pH 1.0)**

1.86 g KCl dissolves in 980 ml of distilled water. Measure the pH and adjust to 1.0 with concentrated HCl. Transfer to a 1 liter volumetric flask and fill to 1 liter with distilled water.

#### **0.4 M Sodium acetate buffer (pH 4.5)**

54.43 g  $\text{CH}_3\text{CO}_2\text{Na}\cdot 3\text{H}_2\text{O}$  dissolves in 960 ml distilled water. Measure the pH and adjust to 4.5 with concentrated HCl. Transfer to a 1 liter volumetric flask and fill to 1 liter with distilled water.

#### **Bisulphite solution**

1 g of potassium metabisulphite ( $\text{K}_2\text{S}_2\text{O}_5$ ) dissolves in 5 ml of distilled water.

#### **1x Folin-Ciocalteu reagent (Fluka 47641)**

10 ml of Folin-Ciocalteu reagent (Fluka 47641) and dilute to 100 ml DI water.

#### **Sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) solution**

7.5 g of sodium carbonate dissolve and make up to 100 ml DI water

#### **Cyanidin 3-glucoside standard**

Stock standard; 1 mg of cyanidin 3-glucoside dissolve in 1 ml 1% aqueous formic acid (1000  $\mu\text{g}/\text{ml}$ ). This solution is dilute to a series of mix working standard solutions of cyanidin 3-glucoside are 10-40  $\mu\text{g}/\text{ml}$ .

#### **Gallic acid standard**

Stock standard; 1 mg of gallic acid is dissolve in 1 ml methanol (1000  $\mu\text{g}/\text{ml}$ ). This solution is dilute to a series of mix working standard solutions of gallic acid are 10-100  $\mu\text{g}/\text{ml}$ .

**Catechin standard**

Stock standard; 10 mg of catechin dissolve in 1 ml methanol (10000 µg/ml). This solution is dilute to a series of mix working standard solutions of catechin are 500-2000 µg/ml.

**Quercetin standard**

Stock standard; 1 mg of quercetin is dissolve in 1 ml methanol (1000 µg/ml). This solution is dilute to a series of mix working standard solutions of quercetin are 50-150 µg/ml.

**Kaempferol standard**

Stock standard; 1 mg of kaempferol is dissolve in 1 ml methanol (1000 µg/ml). This solution is dilute to a series of mix working standard solutions of kaempferol are 50-200 µg/ml.

**Ascorbic acid (vitamin C) standard**

Stock standard; 1 mg of ascorbic acid is dissolve in 1 ml water (1000 µg/ml). This solution is dilute to a series of mix working standard solutions of ascorbic acid are 50-150 µg/ml.

**Dragendroff reagent**

Solution A: 3 g bismuth subnitrate dissolves in 10 ml 25% HCl and make up to 50 ml DI water

Solution B: 30 g KI dissolve and make up to 50 ml DI water

Spray reagent: mix 5 ml solution A + 5 ml solution B + 5 ml HCl 12.5 % and 100 ml DI water

**5% Potassium hydroxide (KOH) reagent**

5 g of potassium hydroxide dissolve in 100 ml 95 % Ethanol

**2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent**

50 mg of 2,2-diphenyl-1-picrylhydrazyl (DPPH) dissolve in 10 ml absolute ethanol

**Natural products-polyethyleneglycol reagent**

Solution A : 1 g of diphenylboric acid- $\beta$ - ethylamino ester dissolve in 100 ml methanol

Solution B : 5 ml polyethyleneglycol- 400 dissolve and make up to 100 ml DI water

Spray reagent: spray solution A and follow by solution B

**Kedde reagent**

Solution A: 3 g of 3,5 dinitrobenzoic acid dissolve and make up to 100 ml DI water

Solution B: 8 g of sodium hydroxide dissolve and make up to 100 ml DI water

Spray reagent: mix 5 ml solution A and 5 ml solution B

**5% Ferric chloride (FeCl<sub>3</sub>) reagent**

5 g of ferric chloride dissolve and make up to 100 ml 95% ethanol

**Sodium carbonate solution**

7.5 g of sodium carbonate dissolve and make up to 100 ml DI water

**0.3% Acetic acid**

3 ml of glacial acetic acid dissolve and make up to 1000 ml DI water and then filter through 0.45 micron filter paper for chromatographic solution.

**1%phosphoric acid/10% acetic acid (glacial)/5% acetonitrile (1:1:1, v/v/v)**

Solution A: 10 ml of phosphoric acid dissolve and make up to 1000 ml DI water

Solution B: 100 ml of glacial acetic acid dissolve and make up to 1000 ml DI water

Solution C: 50 ml of acetonitrile dissolve and make up to 1000 ml DI water

Chromatographic solution: mix 500 ml solution A + 500 ml solution B and 500 ml solution C and then filter through 0.45 micron filter paper.

**$6 \times 10^{-5}$  M 2,2-diphenyl-1-picrylhydrazyl (DPPH)**

2.4 mg of 2,2-diphenyl-1-picrylhydrazyl (DPPH) dissolve in 100 ml methanol

**Liposome**

Weigh 50 mg bovine brain extract (Sigma, B3635) into test tube. Add about 7 glass balls and 10 ml PBS. Sonicate in an ice-water bath until the entire lipid has been suspended and the suspension appears homogeneous and milky in appearance.

**1mM Ferric chloride ( $\text{FeCl}_3$ )**

0.027 g of ferric chloride dissolves in 100 ml DI water

**1 mM Ascorbic acid (vitamin C)**

0.0176 g of ascorbic acid dissolves in 100 ml DI water

**Phosphate buffer saline (PBS)**

1 tablet of PBS dissolves and makes up to 100 ml DI water

**2% Butylate hydroxyl toluene (BHT)**

0.2 g of butylate hydroxyl toluene dissolve in 10 ml absolute ethanol

**1% Thiobarbituric acid (TBA)**

0.5 g of thiobarbituric acid dissolves in 50 ml 50 mM NaOH

**50 mM Sodium hydroxide (NaOH)**

0.2 g of sodium hydroxide dissolves in 100 ml DI water

**1% Hydrochloric acid (HCl)**

68 ml of concentrated hydrochloric acid (37%) make up to 100 ml DI water

#### **Minimum essential medium (MEM)**

9.5 g of Minimum essential powder medium is dissolve in 500 ml distilled water. Add 2.2 g of sodium bicarbonate and dilute to 1000 ml with distilled water. Adjust pH to 7.2-7.4 with 1 N sodium hydroxide or 1 N hydrochloric acid and filter through 0.2 micron membrane filter and keep in sterile bottle.

The complete media is mixture of 400 ml of minimum essential medium (MEM), 40 ml fetal bovine serum and 4 ml penicillin/ streptomycin.

#### **Dulbeco's modified eagle medium (DMEM)**

13.4 g of Dulbeco's modified eagle powder medium is dissolve in 500 ml distilled water. Add 3.7 g of sodium bicarbonate and dilute to 1000 ml with distilled water. Adjust pH to 7.2-7.4 with 1 N sodium hydroxide or 1 N hydrochloric acid and filter through 0.2 micron membrane filter and keep in sterile bottle.

The complete media is mixture of 400 ml of Dulbeco's modified eagle medium (DMEM), 40 ml fetal bovine serum and 4 ml penicillin/ streptomycin.

#### **RPMI medium 1640**

10.4 g of RPMI medium 1640 powder is dissolve in 500 ml distilled water. Add 2.0 g of sodium bicarbonate and dilute to 1000 ml with distilled water. Adjust pH to 7.2-7.4 with 1 N sodium hydroxide or 1 N hydrochloric acid and filter through 0.2 micron membrane filter and keep in sterile bottle.

The complete media is mixture of 400 ml of RPMI medium 1640 and 40 ml fetal bovine serum and 4 ml penicillin/ streptomycin.

#### **0.4% Sulforhodamine B (SRB)**

0.4 g sulforhodamine B dissolve and make up to 100 ml 1% acetic acid (protect light)

#### **40% Trichloroacetic acid (TCA)**

40 g trichloroacetic acid dissolves and makes up to 100 ml DI water

**10 mM Tris buffer (pH 10)**

0.121 g Tris Hydroxymethylaminoethane dissolves in 50 ml DI water and adjusts pH to 10 with 1 N hydrochloric acid.

**5% Hydrochloric acid (HCl)**

50 ml concentrated hydrochloric acid (37%) dissolve and make up to 1000 ml DI water

**Sodium (Na) standard**

Stock standard; 5 ml of 1000 ppm sodium make up to 50 ml 0.2% nitric acid (100 ppm). This solution is dilute to a series of mix working standard solutions of sodium are 0.5-5 ppm.

**Potassium (K) standard**

Stock standard; 5 ml of 1000 ppm potassium make up to 50 ml 0.2% nitric acid (100 ppm). This solution is dilute to a series of mix working standard solutions of potassium are 0.5-5 ppm.

**Calcium (Ca) standard**

Stock standard; 5 ml of 1000 ppm calcium make up to 50 ml 0.2% nitric acid (100 ppm). This solution is dilute to a series of mix working standard solutions of calcium are 0.5-5 ppm.

**Magnesium (Mg) standard**

Stock standard; 5 ml of 1000 ppm magnesium make up to 50 ml 0.2% nitric acid (100 ppm). This solution is dilute to a series of mix working standard solutions of magnesium are 0.5-5 ppm.

**Lead (Pb) standard**

Stock standard; 0.5 ml of 1000 ppm sodium make up to 50 ml 0.2% nitric acid (10 ppm). This solution is dilute to a series of mix working standard solutions of sodium are 50-200 ppb.

**0.1 N Sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) solution**

2.8 ml concentrated sulfuric acid (98%) dissolve and make up to 1000 ml DI water

**40% Sodium hydroxide (caustic soda, NaOH)**

400 g sodium hydroxide dissolves and makes up to 1000 ml DI water

**1.25% Sulfuric acid (H<sub>2</sub>SO<sub>4</sub>)**

12.5 ml concentrated sulfuric acid (98%) dissolve and make up to 1000 ml DI water

**1.25% Sodium hydroxide (NaOH)**

12.5 g sodium hydroxide dissolves and makes up to 1000 ml DI water

**0.2% Nitric acid (HNO<sub>3</sub>)**

2 ml concentrated nitric acid (65%) dissolves and makes up to 1000 ml DI water

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