PRACTICE PROCEDURES FOR MAKING STOCK SOLUTION (4 VERSION STOCK SOLUTION)

A. Tools:

1. Oven
2. Analytical scales
3. Hotplate and magnetic stirrer
4. Beaker glass 1000 ml
5. Bottle
6. Refrigerator
7. Watch glasses
8. Spatula

B. Materials:

1. 18 chemical compounds (according to the listed table of composition of commonly used tissue culture media)
2. Distilled water
3. Alumunium foil
4. Rubber caps
5. Label
6. Tissue

C. Preparation:

1. Prepare the tools and materials needed;
2. Make sure the bottle, beaker glass, spatula have been sterilized in the oven for at least 1 hour with a temperature of at 150^0C, especially for rubber caps, just heat it for a while/just a few minutes so it doesn't melt in the oven;
3. Make sure analytical scales are ready to be used to weigh chemicals;
4. Make sure the hotplate and magnetic stirrer is ready to use to help stir the chemicals that will be dissolved in the distilled water;
5. Make sure the chemical has been appropriately prepared (according to the listed table of the composition of commonly used tissue culture MS media), check before use;
6. Perform the practical procedure below according to each group.

D. Procedures:

<u>STOCK SOLUTION 1 (MACRO, 10 TIMES CONCENTRATION)</u>:

1. Weigh **NH_4NO_3 as much 16,5 gram**, **KNO3 as much 19 gram, $CaCl_2.2H_2O$ as much 4,4 gram, $MgSO_4.7H_2O$ = 3,7 gram, KH_2PO_4 = 1,7 gram;**
2. Prepare a 1000 ml beaker, put in the magnetic stirrer and place it on the hotplate;
3. Put 500 ml of distilled water into a beaker;
4. Press or turn on the button the hotplate at medium speed;
5. Put **NH_4NO_3** into the beaker;

6. Wait a while until it is completely dissolved (if **NH_4NO_3** is difficult to dissolve, heat it at 40-60^{0}C, if it is still difficult to dissolve, add HCl drop by drop until completely dissolved);
7. Put **KNO_3** into the beaker;
8. Wait a while until it is completely dissolved (if **KNO_3** is difficult to dissolve, heat it at 40-60^{0}C, if it is still difficult to dissolve, add HCl drop by drop until completely dissolved);
9. Put **$CaCl_2.2H_2O$** into the beaker;
10. Wait a while until it is completely dissolved;
11. Put **$MgSO_4.7H_2O$** into the beaker;
12. Wait a while until it is completely dissolved;
13. Put **KH_2PO_4** into the beaker;
14. Wait a while until it is completely dissolved;
15. Add distilled water until the volume is exactly 1000 ml;
16. Cool the solution to room temperature (if there is heating treatment);
17. Put/transfer into the culture bottle, cover with a rubber cap;
18. Give a label by writing the name of the stock and the date of making of the stock;
19. Store in the refrigerator;
20. Clean and/or store tools and materials that have been used.

STOCK SOLUTION 2 (MICRO, 100 TIMES CONCENRATION):

1. Weigh **$MnSO_4.4H_2O$ = 1,69 gram, $ZnSO_4.7H_2O$ = 0,86 gram, H_3BO_3 = 0,62 gram, KI = 0,083 gram, $Na_2MoO_4.2H_2O$ = 0,025 gram**, **$CoCl_2.6H_2O$ = 0,0025 gram, $CuSO_4.5H_2O$ = 0,0025 gram;**
2. Prepare a 1000 ml beaker, put in the magnetic stirrer and place it on the hotplate;
3. Put 250 ml of distilled water into a beaker;
4. Press or turn on the button the hotplate at medium speed;
5. Put **$MnSO_4.4H_2O$**, wait a few moments for it to dissolve;
6. Put **$ZnSO_4.7H_2O$**, wait a few moments for it to dissolve;
7. Put **H_3BO_3**, wait a few moments for it to dissolve;
8. Put **KI**, wait a few moments for it to dissolve;
9. Put **$Na_2MoO_4.2H_2O$**, wait a few moments for it to dissolve;
10. Put **$CoCl_2.6H_2O$**, wait a few moments for it to dissolve;
11. Put **$CuSO_4.5H_2O$**, wait a few moments for it to dissolve;
12. Add distilled water until the volume is exactly 1000 ml;
13. Put/transfer into the culture bottle, cover with a rubber cap;
14. Give a label by writing the name of the stock and the date of making of the stock;
15. Store in the refrigerator;
16. Clean and/or store tools and materials that have been used.

STOCK SOLUTION 3 (FE-EDTA, 10 TIMES CONCENTRATION):

1. Weigh **$FeSO_4.7H_2O$ = 0,278 gram dan Na_2EDTA = 0,373 gram**,
2. Prepare a 1000 ml beaker, put in the magnetic stirrer and place it on the hotplate;
3. Put 250 ml of distilled water into a beaker;
4. Press or turn on the button the hotplate at medium speed;

5. Put **Na_2EDTA** into the beaker;
6. Wait a while until it is completely dissolved (if **Na_2EDTA** is difficult to dissolve, heat it at 40-60^{0}C, if it is still difficult to dissolve, add HCl drop by drop until completely dissolved);
7. Put **$FeSO_4.7H_2O$** ino the beaker;
8. Wait a while until it is completely dissolved (if **$FeSO_4.7H_2O$** is difficult to dissolve, heat it at 40-60^{0}C, if it is still difficult to dissolve, add HCl drop by drop until completely dissolved);
9. Add distilled water until the volume is exactly 1000 ml;
10. Cool the solution to room temperature (if there is heating treatment);
11. Put/transfer into the culture bottle, cover with a rubber cap;
12. Wrap the bottle in alumunium foil tightly;
13. Give a label by writing the name of the stock and the date of making of the stock;
14. Store in the refrigerator;
15. Clean and/or store tools and materials that have been used.

STOCK SOLUTION 4 (VITAMIN, 100 TIMES CONCENTRATION):

1. Weigh **Thyamine-HCl = 0,04 gram, Glycine = 0,2 gram, Nicotinic-acid = 0,005 gram** and **Pyridoxine = 0,005 gram;**
2. Prepare a 1000 ml beaker, put in the magnetic stirrer and place it on the hotplate;
3. Put 250 ml of distilled water into a beaker;
4. Press or turn on the button the hotplate at medium speed;
5. Put **Thyamine-HCl**, wait a few moments for it to dissolve;
6. Put **Glycine**, wait a few moments for it to dissolve;
7. Put **Nicotinic-acid**, wait a few moments for it to dissolve;
8. Put **Pyridoxine**, wait a few moments for it to dissolve;
9. Add distilled water until the volume is exactly 1000 ml;
10. Put/transfer into the culture bottle, cover with a rubber cap;
11. Give a label by writing the name of the stock and the date of making of the stock;
12. Store in the refrigerator;
13. Clean and/or store tools and materials that have been used.

PRACTICE PROCEDURES FOR MAKING STOCK SOLUTIONS (6 VERSION STOCK SOLUTION)

A. Tools:

1. Oven
2. Analytical scales
3. Hotplate and magnetic stirrer
4. Beaker glass 1000 ml
5. Bottle
6. Refrigerator
7. Watch glasses
8. Spatula

B. Materials:

1. 18 chemical compounds (according to the listed table of composition of commonly used tissue culture media)
2. Distilled water
3. Alumunium foil
4. Rubber caps
5. Label
6. Tissue

C. Preparation:

1. Prepare the tools and materials needed;
2. Make sure the culture bottle, beaker glass, spatula have been sterilized in the oven for at least 1 hour with a temperature of at 150^0C, especially for rubber caps, just heat it for a while/just a few minutes so it doesn't melt in the oven;
3. Make sure analytical scales are ready to be used to weigh chemicals;
4. Make sure the hotplate and magnetic stirrer is ready to use to help stir the chemicals that will be dissolved in the distilled water;
5. Make sure the chemical has been prepared properly (according to the listed table of composition of commonly used tissue culture MS media), check before use.

D. Procedures:

<u>STOCK SOLUTION 1 (10 TIMES CONCENTRATION)</u>:

1. Weigh **NH_4NO_3 sebanyak 16,5 gram** and **KNO3 sebanyak 19 gram**;
2. Prepare a 1000 ml beaker, put in the magnetic stirrer and place it on the hotplate;
3. Put 250 ml of distilled water into a beaker;
4. Press or turn on the button the hotplate at medium speed;
5. Put **NH_4NO_3** into the beaker;
6. Wait a while until it is completely dissolved (if **NH_4NO_3** is difficult to dissolve, heat it at 40-60^0C, if it is still difficult to dissolve, add HCl drop by drop until completely dissolved);
7. Put **KNO_3** into the beaker;

8. Wait a while until it is completely dissolved (if **KNO_3** is difficult to dissolve, heat it at 40-60^{0}C, if it is still difficult to dissolve, add HCl drop by drop until completely dissolved);
9. Add distilled water until the volume is exactly 1000 ml;
10. Cool the solution to room temperature (if there is heating treatment);
11. Put/transfer into the culture bottle, cover with a rubber cap;
12. Give a label by writing the name of the stock and the date of making of the stock;
13. Store in the refrigerator;
14. Clean and/or store tools and materials that have been used.

STOCK SOLUTION 2 (10 TIMES CONCENTRATION):

1. Weigh **$CaCl_2.2H_2O$ as much 4,4 gram;**
2. Prepare a 1000 ml beaker, put in the magnetic stirrer and place it on the hotplate;
3. Put 250 ml of distilled water into a beaker;
4. Press or turn on hotplate at medium speed;
5. Put **$CaCl_2.2H_2O$** into the beaker;
6. Wait a while until it is completely dissolved;
7. Add distilled water until the volume is exactly 1000 ml;
8. Put/transfer into the culture bottle, cover with a rubber cap;
9. Give a label by writing the name of the stock and the date of making of the stock;
10. Store in the refrigerator;
11. Clean and/or store tools and materials that have been used.

STOCK SOLUTION 3 (10 TIMES CONCENTRATION):

1. Weigh **$MgSO_4.7H_2O$ = 3,7 gram, KH_2PO_4 = 1,7 gram**, **$MnSO_4.4H_2O$ = 0,169 gram, $ZnSO_4.7H_2O$ = 0,086 gram, H_3BO_3 = 0,062 gram.**
2. Prepare a 1000 ml beaker, put in the magnetic stirrer and place it on the hotplate;
3. Put 250 ml of distilled water into a beaker;
4. Press or turn on the button the hotplate at medium speed;
5. Put **$MgSO_4.7H_2O$**, wait a few moments for it to dissolve;
6. Put **KH_2PO_4**, wait a few moments for it to dissolve;
7. Put **$MnSO_4.4H_2O$**, wait a few moments for it to dissolve;
8. Put **$ZnSO_4.7H_2O$**, wait a few moments for it to dissolve;
9. Put **H_3BO_3**, wait a few moments for it to dissolve;
10. Add distilled water until the volume is exactly 1000 ml;
11. Put/transfer into the culture bottle, cover with a rubber cap;
12. Give a label by writing the name of the stock and the date of making of the stock;
13. Store in the refrigerator;
14. Clean and/or store tools and materials that have been used.

STOCK SOLUTION 4 (40 TIMES CONCENTRATION):

1. Weigh **KI = 0,0332 gram, $Na_2MoO_4.2H_2O$ = 0,01 gram**, **$CoCl_2.6H_2O$ = 0,001 gram, $CuSO_4.5H_2O$ = 0,001 gram,**
2. Prepare a 1000 ml beaker, put in the magnetic stirrer and place it on the hotplate;
3. Put 250 ml of distilled water into a beaker;
4. Press or turn on the hotplate at medium speed;

5. Put **KI**, wait a few moments for it to dissolve;
6. Put **$Na_2MoO_4.2H_2O$**, wait a few moments for it to dissolve;
7. Put **$CoCl_2.6H_2O$**, wait a few moments for it to dissolve;
8. Put **$CuSO_4.5H_2O$**, wait a few moments for it to dissolve;
9. Add distilled water until the volume is exactly 1000 ml;
10. Put/transfer into the culture bottle, cover with a rubber cap;
11. Give a label by writing the name of the stock and the date of making of the stock;
12. Store in the refrigerator;
13. Clean and/or store tools and materials that have been used.

STOCK SOLUTION 5 (10 TIMES CONCENTRATION):

1. Weigh **$FeSO_4.7H_2O$ = 0,278 gram** and **Na_2EDTA = 0,373 gram**,
2. Prepare a 1000 ml beaker, put in the magnetic stirrer and place it on the hotplate;
3. Put 250 ml of distilled water into a beaker;
4. Press or turn on the button the hotplate at medium speed;
5. Put **Na_2EDTA** into the beaker;
6. Wait a while until it is completely dissolved (if **Na_2EDTA** is difficult to dissolve, heat it at 40-60^{0}C, if it is still difficult to dissolve, add HCl drop by drop until completely dissolved);
7. Put **$FeSO_4.7H_2O$** into the beaker;
8. Wait a while until it is completely dissolved (if **$FeSO_4.7H_2O$** is difficult to dissolve, heat it at 40-60^{0}C, if it is still difficult to dissolve, add HCl drop by drop until completely dissolved);
9. Add distilled water until the volume is exactly 1000 ml;
10. Cool the solution to room temperature (if there is heating treatment);
11. Put/transfer into the culture bottle, cover with a rubber cap;
12. Wrap the bottle in alumunium foil tightly;
13. Give a label by writing the name of the stock and the date of making of the stock;
14. Store in the refrigerator;
15. Clean and/or store tools and materials that have been used.

STOCK SOLUTION 6 (VITAMIN, 100 TIMES CONCENTRATION):

1. Weigh **Thyamine-HCl = 0,04 gram, Glycine = 0,2 gram, Nicotinic-acid = 0,005 gram** and **Pyridoxine = 0,005 gram;**
2. Prepare a 1000 ml beaker, put in the magnetic stirrer and place it on the hotplate;
3. Put 250 ml of distilled water into a beaker;
4. Press or turn on the hotplate at medium speed;
5. Put **Thyamine-HCl**, wait a few moments for it to dissolve;
6. Put **Glycine**, wait a few moments for it to dissolve;
7. Put **Nicotinic-acid**, wait a few moments for it to dissolve;
8. Put **Pyridoxine**, wait a few moments for it to dissolve;
9. Add distilled water until the volume is exactly 1000 ml;
10. Put/transfer into the culture bottle, cover with a rubber cap;
11. Give a label by writing the name of the stock and the date of making of the stock;

12. Store in the refrigerator;
13. Clean and/or store tools and materials that have been used.