

BIOENGINEERING LABORATORY WORK PLAN

1. General Information

Name of researchers: ZOU Hang

Name of project supervisor: Tang Benzhong

Project code or title: Study the application of AIEgens in bio-imaging field.

Proposed location and start date: BIEN Lab, ASAP

2. Experiment /Project Description

- Flow cytometry will be used in the study of the toxicity of the AIEgens.
- Mammalian cell (HepG2, A549, MCF cell) culture method will be used to check the results of the fluorescence staining, the toxicity, bio-application, or drug effect of the AIEgens conjugated bio-materials.

3. Equipment List

Process	Apparatus
Mammalian Cell Culture	37 centigrade water bath, Biosafety Cabinet, Inverted microscope, CO ₂ incubator, 96-well tissue culture plate, Pasteur pipette, 75cm ² flask, tissue culture dish, digital camera, Plate reader
Cell staining	Pipette, Biosafety Cabinet, Incubator
Toxicity	Use flow cytometry

4. Experimental Procedures

4.1 Mammalian Cell Culture

1. Pre-warm all solutions at 37 centigrade before use.
2. Turn on the UV of BSC for 15 to 30 min and then switch it off.
3. Turn on the BSC for at least 15 min before use.
4. Spray every reagent with 70% ethanol and put them into the BSC.
5. Obtain a cell flask with about 80-90% cell confluence (Check under microscope).
6. Remove old medium by a Pasteur pipet connected to a pump.
7. Wash the cell with 10 ml PBS and then remove it.
8. Add 1.5ml 1trypsin and then put back to incubator for 3 min, then take it out.
9. Add fresh MEM 3-5 ml by glass pipet, and then mix up.
10. Count cell concentration with MARIEBFELD hemocytometry.
11. Dispart 1-3 ml medium containing cells to a new flask containing fresh medium to make sure that the cell concentration is 75000/ml.
12. Add 100 ul diluted cell into each well of the 96-well culture plate.
13. Incuate cells at 37 centigrade and 5% carbon dioxide for later use.

4.2 Cell Staining

Dissolve AIE fluorescent molecules into the culture medium with cells. After 15 minutes, observe the cells with fluorescent microscope.

4.3 Toxicity

HepG2 cells were stained with AIE solution for several hours. Cell apoptosis was measured by Annexin V-FITC/PI Apopto-sis Detection Kit. Cells were digested with 0.05% Trypsin and stained with annexin V-FITC and propidium iodide in the dark at room temperature. After treatment at a given time, apoptotic cells were measured by a flow cytometer.

5. Operation Condition

- CO² incubator temperature: 37 centigrade
- CO² concentration of the CO² incubator: 5%
- Pre-warm solutions temperature: 37 centigrade (water bath temperature)

6. Service List

Water: Tap water, distilled water

7. Chemical List

Chemical	Quantity per experiment	MSDS attached
Phosphate Buffered Saline	20ml	Yes
DMSO	1ml	Yes
Medium	30ml	Yes
AIE Molecules	0.001g	No
formamide	0.9ml	Yes
glycerol	6ml	Yes
Annexin V-FITC	5ul	Yes
PI (propidium iodide)	5ul	Yes
Trypsin	1ml	Yes

8. Summary of Relevant Hazards and Incompatibilities List

Chemical	Concentration	Summary of Relevant Hazards	Incompatibilities
DMSO	100%	May cause irritation Liver and kidney damage could occur.	Oxidizers, acyl halides, active halogen compounds and strong acids
Medium	1x	Not toxic	N/A
AIE Molecules	100%	Not toxic	N/A

formamide	0.9ml	May cause harm to the unborn child.	Strong oxidizing agents, acids, bases
glycerol	6ml	N/A	Oxidizing agents, strong acids, acetic anhydride, isocyanates, aliphatic amines, potassium permanganate, caustics
Annexin V-FITC	5ul	Excitability. May cause harm to the skin	Causticity
PI (propidium iodide)	5ul	Excitability. May cause harm to the skin.	Causticity
Trypsin	0.05%	May cause harm to the skin.	Strong oxidizing agents

9. Waste Disposal and Handling

- All biological waste will be autoclaved at 121°C for 60 minutes before disposal.
- The liquids contact with cells will be disinfected by bleach.

10. Assessment of Significant Risks

The main risk and hazard in this experiment are mainly from the employment and use of different chemicals to treat the mammalian cells, waste cells, and solvent. Attention and care must be taken when doing experiment. Also suitable gloves, lab coat must be worn to prevent the contact of chemicals with the human body.

11. Safety precautions

11.1 Personal Protection

In order to ensure the safety of the researcher who carries out the experiment, appropriate personal protective equipment must be employed. For example, lab coat is required as it provides a front line protection against various dangers. Protective gloves must be used when handling the above mentioned substances.

11.2 Equipment and Safety Training

Before carrying out any experiments in the laboratory, the researcher must be trained so that proper operation of equipment used in the experiment can be ensured. In the experiment-training program, the researcher must also learn how to handle emergency situation associated with the equipment in such case as the broken down and the failure of the equipment.

Moreover, safety training to the researcher is also necessary so that the researcher can understand thoroughly the proper procedures in handling chemical waste disposal and dealing with high pressure etc, thus carrying out suitable measures to prevent the happening of accidents.

11.3 General Laboratory Safety Rules and Administrative Control

The general laboratory safety rule must be obeyed. In addition, regular monitoring and maintenance of the chemicals equipment and personal protective equipment must be implemented so as to minimize the chance of accidents.

12. Action in Case of Abnormal or Emergency Situations

12.1 Service failure

In case of the failure and damage of the experimental instrument, switch it off and cut the power supply immediately and then inform the technician before taking any further actions.

12.2 Fire or Explosion

In case of a fire or explosion, the room should be evacuated immediately and the danger alarm should be activated in order to inform the HSEO and the campus security or inform them by dialing 8999 at a safe place.

12.3 Loss of Containment or Spillage

For small spillage, inform the technician immediately and clean up the area carefully adhering to the corresponding safety procedures.

For large spillage or loss of containment, the room should be evacuated immediately and the danger alarm should be activated in order to inform the HSEO and the campus security or inform them by dialing 8999 at a safety place.

12.4 Other Possible Abnormal Situation

N/A

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Signature 邹航

Date 05.06.2017

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Signature [Signature]

Date _____