Hemolysis Test Protocol

Preparation:

Red blood cells**:**

1. Draw blood from the rat.
2. Waiting for red blood cell (RBC) precipitation in the bottom of the blood collection tube.
3. Making 8 mL of 0.5% v/v RBC suspension: 40 µL of RBC is added to 8 mL of Phosphate buffered saline (PBS) buffer in a 15mL tube. Repeat three times in three different 15mL tubes.

Test Procedure:

1. Take a 96-well plate and transfer 150 µL of peptide solution in PBS in row A (one peptide four columns).
2. Using a multichannel pipette to transfer 75 µL of PBS to all the wells from B1 to E12.
3. Negative control: transfer 75 µL of PBS in the wells F1 to F12.
4. Positive control: transfer 75 µL of 1% triton x-100 in the wells G1 to G12.
5. Blank: transfer 150 µL of PBS in the wells H1 to H12.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Pep1	Pep1	Pep1	Pep1	Pep2	Pep2	Pep2	Pep2	Pep3	Pep3	Pep3	Pep3
B	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS
C	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS
D	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS
E	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS
F	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
G	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
H	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank

6. Transfer 75 µL from row A to row B, mix five times.
7. Transfer 75 µL from row B to row C, mix five times.
8. Transfer 75 µL from row C to row D, mix five times.
9. Transfer 75 µL from row D to row E, mix five times.
10. After mixing, discard 75 µL from row E.
11. Using a multichannel pipette to transfer 75 µL of RBC suspension to all the wells from A1 to G12.

Incubating and Reading Absorbance:

1. Place in the incubator at 37 °C for 1 hour.
2. Centrifuge the plates at 1000 xg for 10 min.
3. Transfer 60 µL of supernatant from each well into a new flat-bottom 96-well ELISA plate.
4. Read absorbance at λ=414 nm.