Sheep Hemagglutination Kit
(KPA-3913)

The Sheep Hemagglutination Kit (KPA-3913) is an easy-to-use kit that provides the positive control, erythrocytes, and an round-bottom ELISA plate for rapid detection of infections for research use. Hemagglutination assays can be performed with agglutinating virus samples or samples containing lectin and/or glycoproteins.

I. Kit Components

Component	Catalog #
10X PBS pH 7.2	MB-008-0010
Anti-SHEEP Red Blood Cell (RBC)(RABBIT) Antibody	213-4139-0002
96-Well Round Bottom Microwell Plate with Lid	N/A
Sheep RBC 10% Washed Pooled Cells	R405-0005

II. Reagents and Materials Required but Not Provided

1. 1 mL pipette and tips
   Note: Cut tip so pipetting does not disrupt the RBCs.
2. 200 µL pipette and tips
   Note: Cut tip so pipetting does not disrupt the RBCs.
3. Millipore Stericup® or similar 0.22 µm filter
4. Molecular Biology Grade UltraPure Water (#MB-010-0100) or 0.22 µm filtered water
5. Kimwipes® or similar cleaning products
6. 15 mL conical tubes and assorted Eppendorf vials or similar
7. 5 mL syringe and needle

III. Reagent Preparation

1X PBS

1. Bring 10X PBS pH 7.2 to room temperature.
2. Measure 90 mL of DI water from faucet into a 100 mL graduated cylinder.
3. Pour 10 mL of 10X PBS into the graduated cylinder for a total volume of 100 mL.
4. Mix well and sterilize using a 0.22 µm filter.

0.5% RBC Stock

1. Gently invert the 10% stock of RBCs 10 times, gently mixing.
2. Add 1.9 mL of 1X PBS working solution to a conical tube.
3. Using a 1 mL pipette with a cut tip, add 100 µL RBCs to 1X PBS working solution in tube.
4. Gently invert 5 times to mix gently.

Anti-SHEEP RBC Antibody

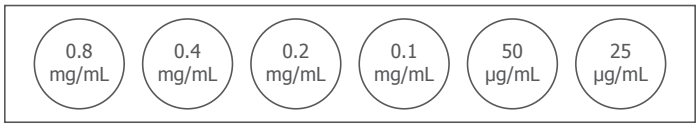
1. Sanitize bench space.
2. Reconstitute the antibody by inserting 200 µL of ultrapure water or equivalent with a needle-tipped syringe into the rubber stopper.
   Note: If needle is not available, very carefully insert the water into the vial without taking out the stopper. This will prevent any loss of lyophilized powder.
3. Vortex and let sit at room temperature until the solution is clear. The final antibody concentration will be 10 mg/mL.

IV. Procedure

1. Prepare and label vials for the number of dilutions desired.
2. Add 414 µL of 1X PBS to first vial.
3. To subsequent vials, add 220 µL of 1X PBS to the planned target final concentration.

4. Add 36 µL of the antibody working solution to the first vial.
5. Gently pipette mix and vortex. The antibody concentration will be 0.8 mg/mL.
6. Remove 220 µL of the first vial to the second.
7. Gently pipette mix and vortex
   Note: Use same tip.
8. Continue serial diluting in sequential order of the pre-labeled vials.
9. Add 50 µL of 1X PBS to the control well in duplicate.
10. Add 50 µL of each prepared dilution into associated wells using the figure below as a guide.

Figure 1. Dilution Schematic



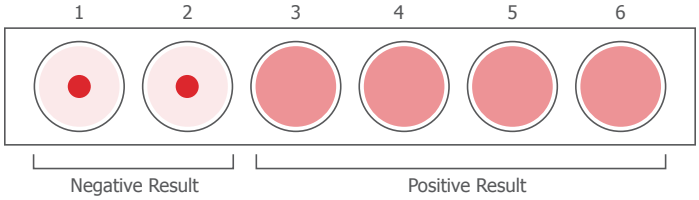
11. Add 50 µL of 0.5% RBC stock solution to each well using a 200 µL pipette with cut tip.
12. Gently mix the wells by moving the plate in circles on a flat surface for 10 seconds.
13. Let the plate incubate for 90 minutes at room temperature.
    Note: It is recommended to cover with a lid.
14. Image plate using a lit background with phone camera or equivalent.

V. Reading Results

Wells with the PBS-only control will form a red button or dot shape at the bottom of the well. This negative result will also occur in end-user samples devoid of hemagglutinating substances.

Wells with the sheep RBCs and agglutinating antibody will generate a uniform red color or diffuse suspension, as will a positive end-user sample that contains hemagglutinating substances, such as virus particles.

Figure 2. Reading Results



VI. Additional Information

In a viral hemagglutination assay, a virus dilution (e.g. 2-fold from 1:4 to 1:4096) will be applied to an RBC dilution (e.g. 0.05% -0.1%) for 30 minutes to an hour at room temperature or 4°C. After which, the lattice formation is observed and the titer of a hemagglutination assay is determined by the last viable "lattice" structure found.

On the other hand, a Hemagglutination-Inhibition (HAI) assay will involve titration of the viral hemagglutination with an antiviral antibody (often from serum of human or animal infected with that virus) for inhibition of hemagglutination (i.e. neutralization of virus). HAI is one of the most commonly used methods to quantify immunity from influenza and other respiratory viral disease vaccines. It is also considered the gold standard as it correlates to vaccination mediated protection.