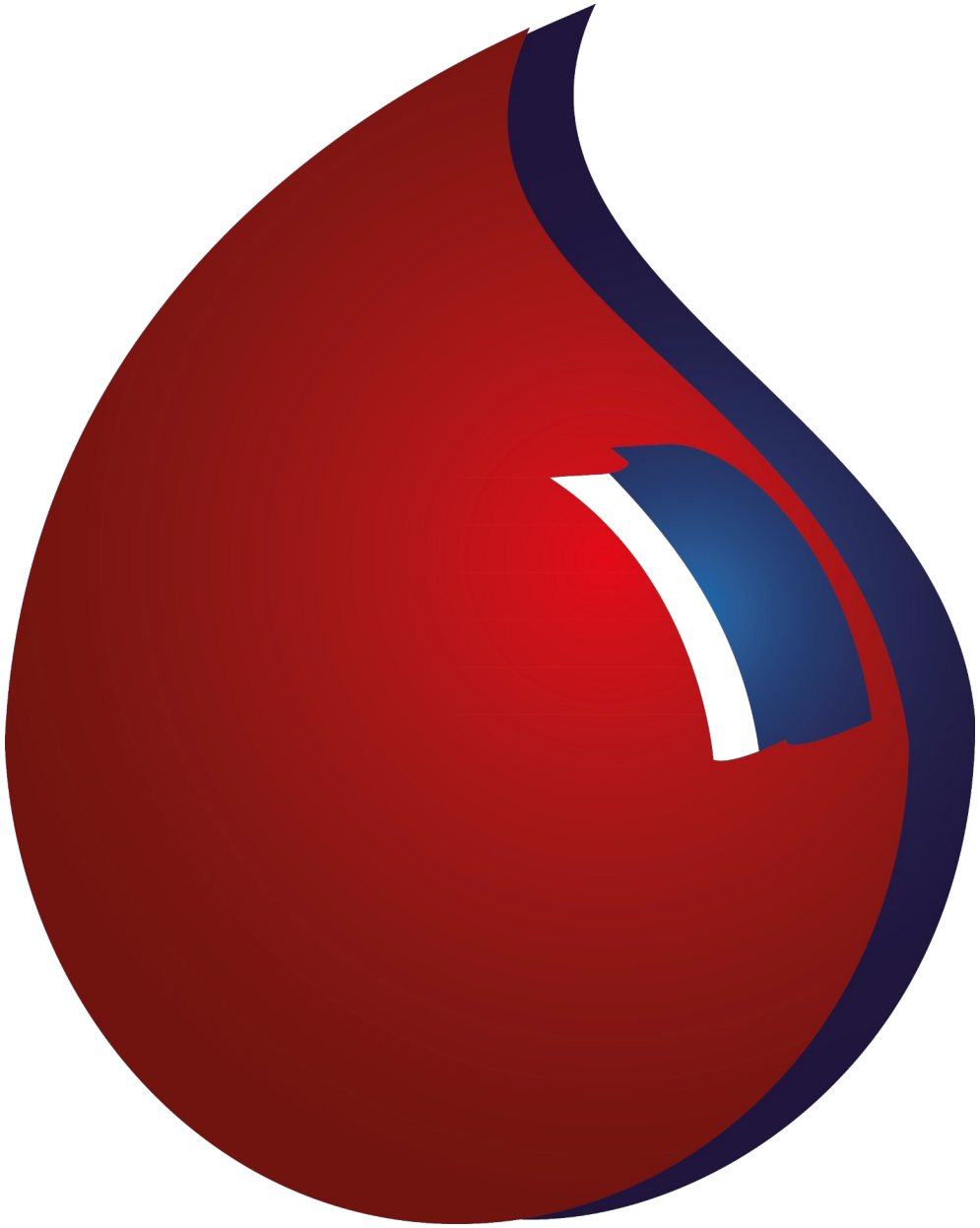

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Haemolysis Assay for Solutes

Kit Manual

www.HaemoScan.com

Product number: K007
Version: 02-11-2026

Introduction

Determination of haemolytic activity is a requirement during the development of new pharmaceuticals and other solutes. Erythrocyte lysis may be caused by the compound itself, by excipients or impurities, or by the composition of the formulation (e.g. pH or osmolality). In vivo, haemolysis may cause (severe) adverse events, owing to anaemia and the release of iron and other bioactive substances.

This assay is based on direct contact between a solute and an erythrocyte suspension, and on the release of haemoglobin, which is measured spectrophotometrically.

Principle of the Test

An erythrocyte suspension is incubated with the solute for 1 hour at 37 °C. After incubation, the samples are centrifuged to obtain a supernatant. The free haemoglobin concentration in the supernatant is measured spectrophotometrically at 415 nm. Alternatively, a wavelength of 540 nm can be used.

The kit can be used to screen solutes and pharmaceuticals at their highest intended concentration. Compounds that are found to induce haemolysis can subsequently be tested in a dose–response experiment to determine the EC50, i.e. the concentration that causes 50% haemolysis.

Precautions

- The kit is intended for research use only. It is not intended for diagnostic or therapeutic procedures.
- Store the kit at 4–6 °C.
- Do not use the kit beyond its expiry date.
- Do not combine reagents from Haemolysis Assay kits with different lot numbers.
- The erythrocytes are of human origin. Each donation was tested and found negative for anti-HIV-1/2, HBsAg, anti-HCV, syphilis, HIV-1 RNA, HBV DNA and HCV DNA. As no test method can completely exclude the presence of infectious agents, handle the erythrocytes as potentially infectious material (biosafety level 2).
- The Lysis fluid and the Reference sample contain Triton X-100, which may cause endocrine disruption in the environment; the Reference sample is also harmful to aquatic life with long-lasting effects. Avoid release to the environment and collect spillage.

- Dispose of the kit components, samples and contaminated materials as biological (potentially infectious) waste in accordance with local and national regulations. For further information, please refer to the Safety Data Sheet (SDS), available at www.haemoscan.com.
- Wear disposable nitrile gloves, a laboratory coat and safety glasses. Do not pipette by mouth.
- Use disposable pipette tips throughout the procedure to avoid contamination of reagents.

Contents of the Kit

• Erythrocyte concentrate (human)	1.5 mL	1 tube
• Assay buffer (0.9% NaCl)	100 mL	1 bottle
• Lysis fluid (positive control)	3 mL	1 bottle
• Reference sample (Triton X-100, 0.625%)	0.5 mL	1 tube

Additional Materials and Equipment

The following materials and equipment are required but are not provided with the kit:

- Calibrated adjustable pipettes with disposable tips
- Incubator at 37 °C
- Microplate reader capable of measuring at 415 nm
- Microcentrifuge with 1.5 mL microcentrifuge tubes, or a centrifuge with microplate rotor, round-bottom and flat-bottom 96-well microplates and microplate sealing film

Test Procedure

Note: The protocol below describes the preparation of erythrocyte suspension for the maximum number of samples (2 × 96 wells). Smaller batches of samples can be analysed with a correspondingly smaller volume of erythrocyte suspension; calculate the required volume accordingly. Store unused erythrocytes as concentrate at 4–6 °C.

Reagent Preparation

Erythrocyte suspension:

1. Add 1 mL of Erythrocyte concentrate to 5 mL of Assay buffer and mix gently by end-over-end inversion of the tube.
2. Centrifuge the tube for 5 minutes at 400 × g and aspirate or decant the supernatant. Repeat this washing step if the supernatant is not colourless.
3. Resuspend the pellet in 20 mL of Assay buffer. This is sufficient for 2 microplates.
4. Mix 50 µL of the erythrocyte suspension with 150 µL of Lysis fluid. Measured in a flat-bottom microplate, this mixture should give an OD_{415} between 0.500 and 0.600. If the OD_{415} is above 0.600, add 1 mL of Assay buffer and repeat the measurement. If the OD_{415} is below 0.500, add more Erythrocyte concentrate and repeat the measurement.

Note: Instead of the Assay buffer, the buffer in which the sample is dissolved or suspended may be used.

Test samples: For the screening procedure, dissolve or suspend the samples in Assay buffer, or in another buffer of the user's choice, at the highest concentration of the intended application. For the EC50 procedure, prepare a serial dilution of each sample; the dilution factor is at the user's discretion.
The negative control is (Assay) buffer; the positive control is Lysis fluid.
Reference sample: Dilute the Reference sample 10-fold in Assay buffer, resulting in a concentration of 0.0625% Triton X-100. Then prepare a six-point serial dilution with a dilution factor of 4 (0.0625% to 0.000061%).

Assay Procedure

1. Pipette 100 µL of each test sample, reference sample and control into a round-bottom microplate.
2. Add 100 µL of erythrocyte suspension to each well.
3. Cover the plate with microplate sealing film.
4. Incubate for 1 hour at 37 °C.
5. Centrifuge the plate for 10 minutes at 400 × g to pellet the intact erythrocytes.
6. Transfer 100 µL of each supernatant to a well of a flat-bottom microplate.
7. Measure the OD at 415 nm.

Calculations

1. Subtract the mean OD_{415} of the negative control from the OD_{415} of all test samples, reference samples and the positive control.
2. Calculate the percentage haemolysis for each sample and concentration as OD_{415} (sample) / OD_{415} (positive control) × 100%.
3. Plot the percentage haemolysis as the dependent variable (y) against the natural logarithm (ln) of the concentration as the independent variable (x). See Figure 2.
4. Determine the slope (a) and intercept (b) of the best-fit line:
   - $y = a \cdot \ln(x) + b$
5. Solve for x:
   - $x = \exp((y - b) / a)$
6. For the EC50, y = 50%:
   - $EC50 = \exp((50\% - b) / a)$

An example calculation spreadsheet is available on request.

Note: Because the data are expected to follow a sigmoidal curve, a four-parameter logistic (4PL) fit is better suited, although less convenient in spreadsheet programs. With the linear approximation, use the concentrations that give approximately 10–90% haemolysis.

	1	2	3	4	5	6	7	8	9	10	11	12
A	$\frac{1}{10^{-4}}$	$\frac{1}{10^{-5}}$	$\frac{1}{10^{-6}}$	$\frac{1}{10^{-7}}$	$\frac{1}{10^{-8}}$	$\frac{1}{10^{-9}}$	$\frac{9}{10^{-4}}$	$\frac{9}{10^{-5}}$	$\frac{9}{10^{-6}}$	$\frac{9}{10^{-7}}$	$\frac{9}{10^{-8}}$	$\frac{9}{10^{-9}}$
B	$\frac{2}{10^{-4}}$	$\frac{2}{10^{-5}}$	$\frac{2}{10^{-6}}$	$\frac{2}{10^{-7}}$	$\frac{2}{10^{-8}}$	$\frac{2}{10^{-9}}$	$\frac{10}{10^{-4}}$	$\frac{10}{10^{-5}}$	$\frac{10}{10^{-6}}$	$\frac{10}{10^{-7}}$	$\frac{10}{10^{-8}}$	$\frac{10}{10^{-9}}$
C	$\frac{3}{10^{-4}}$	$\frac{3}{10^{-5}}$	$\frac{3}{10^{-6}}$	$\frac{3}{10^{-7}}$	$\frac{3}{10^{-8}}$	$\frac{3}{10^{-9}}$	$\frac{11}{10^{-4}}$	$\frac{11}{10^{-5}}$	$\frac{11}{10^{-6}}$	$\frac{11}{10^{-7}}$	$\frac{11}{10^{-8}}$	$\frac{11}{10^{-9}}$
D	$\frac{4}{10^{-4}}$	$\frac{4}{10^{-5}}$	$\frac{4}{10^{-6}}$	$\frac{4}{10^{-7}}$	$\frac{4}{10^{-8}}$	$\frac{4}{10^{-9}}$	$\frac{12}{10^{-4}}$	$\frac{12}{10^{-5}}$	$\frac{12}{10^{-6}}$	$\frac{12}{10^{-7}}$	$\frac{12}{10^{-8}}$	$\frac{12}{10^{-9}}$
E	$\frac{5}{10^{-4}}$	$\frac{5}{10^{-5}}$	$\frac{5}{10^{-6}}$	$\frac{5}{10^{-7}}$	$\frac{5}{10^{-8}}$	$\frac{5}{10^{-9}}$	$\frac{13}{10^{-4}}$	$\frac{13}{10^{-5}}$	$\frac{13}{10^{-6}}$	$\frac{13}{10^{-7}}$	$\frac{13}{10^{-8}}$	$\frac{13}{10^{-9}}$
F	$\frac{6}{10^{-4}}$	$\frac{6}{10^{-5}}$	$\frac{6}{10^{-6}}$	$\frac{6}{10^{-7}}$	$\frac{6}{10^{-8}}$	$\frac{6}{10^{-9}}$	$\frac{14}{10^{-4}}$	$\frac{14}{10^{-5}}$	$\frac{14}{10^{-6}}$	$\frac{14}{10^{-7}}$	$\frac{14}{10^{-8}}$	$\frac{14}{10^{-9}}$
G	$\frac{7}{10^{-4}}$	$\frac{7}{10^{-5}}$	$\frac{7}{10^{-6}}$	$\frac{7}{10^{-7}}$	$\frac{7}{10^{-8}}$	$\frac{7}{10^{-9}}$	REF 1	REF 2	REF 3	REF 4	REF 5	REF 6
H	$\frac{8}{10^{-4}}$	$\frac{8}{10^{-5}}$	$\frac{8}{10^{-6}}$	$\frac{8}{10^{-7}}$	$\frac{8}{10^{-8}}$	$\frac{8}{10^{-9}}$	NC	NC	NC	PC	PC	PC

Figure 1. Example 96-well plate layout for EC50 dose–response curves. 1–14: test samples at six concentrations; REF 1–6: Reference sample dilution series (REF 1 = 0.0625% Triton X-100); NC/PC: negative/positive control.

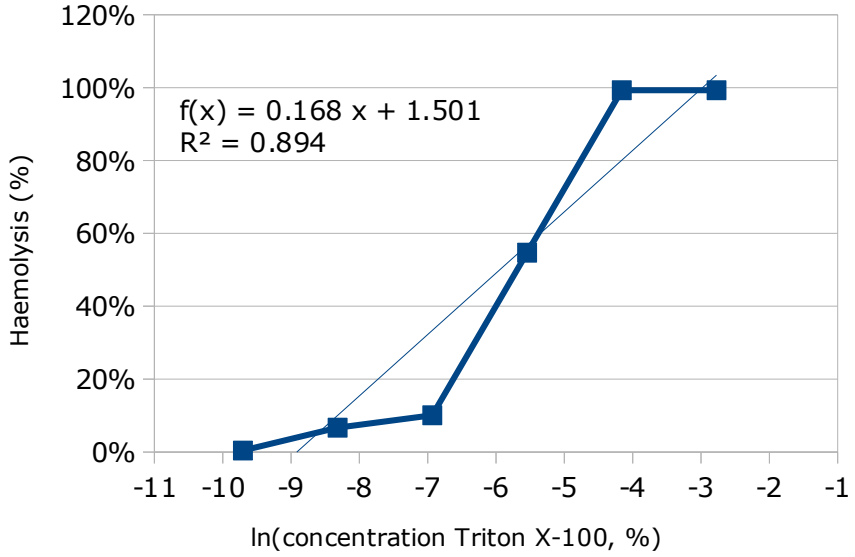

Figure 2. Example EC50 calculation (Reference sample): EC50 = exp((0.50 − 1.501) / 0.168) = 0.0026% Triton X-100.