Hemolysis Assay

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Abstract

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Protocol

Step 1.

Wash and concentrate blood, prepare 2% blood solution

Add 3 mL rabbit blood to 14 mL PBS pH 5.7 and mix

Centrifuge 10 min 500g 4C

Remove supernatant

Repeat steps for a total of 4 washes

After the final wash, remove 200 uL blood from the bottom of the vial and add to 9.8 mL PBS pH 5.7

Step 2.

Prepare endosomal disruptor solutions

Prepare 100 uL solutions of endosomal disruptors at different concentrations (Ex serial 10x dilutions of TritonX from 15 mg/mL to .0015 mg/mL).

Step 3.

Add blood to endosomal disruptors and incubate

Add 50 uL blood solution to 100 uL endosomal disruptor solutions

Incubate at 37C for 30 min, starting timer as soon as last solution added.

Step 4.

Make positive control

Make a positive control (known lysis) by adding 50 uL blood solution to 100 uL diH2O and freeze-thaw cycling 3 times (freeze in -80C freezer)

Step 5.

Centrifuge and place in plate
Centrifuge in tabletop centrifuge at 2500g for 6 min

Carefully collect 75 uL of supernatant from each tube and place in 96 well plate

**Solutions bubble easily so be very carefully not to introduce any air

**Disrupting bottom pellet creates false positives

Step 6.

Take absorbance
Take absorbance at 541 nm

Can also do absorbance scan if worried about side reactions