Customer Information Sheet

AGGFix (catalogue no. PSR-002A/2B)

For fixation of platelet aggregates and other blood cell conjugates prior to immunofluorescence staining and flow cytometric analysis.

The fixative is supplied as two solutions – AGGFixA and AGGFixB, which used consecutively, provide fixation and long-term stability of the sample under investigation. Use AGGFixA at a ratio of 1 volume of AGGFixA to 3 volumes of sample requiring fixation, then, after 10 minutes but no longer than 30 minutes, dilute an aliquot of the fixed sample 10-fold with AGGFixB. Two-step fixation – with AggFixA and further dilution with AggFixB – is crucial to achieving long-term stability. This procedure has been tested for quantification of platelet aggregates in human whole blood and platelet-rich plasma (PRP). AGGFix may also be suitable for quantification of platelet-leucocyte conjugates in whole blood, and for use with species other than humans.

INTENDED USE

AGGFix was developed for the quantification of single platelets and platelet aggregates in whole blood and platelet-rich plasma (PRP). The intention was to fix the single platelets and platelet aggregates and render them stable for up to 9 days. This enables samples to be fixed remotely and transported to a central laboratory for quantitative analysis using immunofluorescence staining and flow cytometry.

SUMMARY AND EXPLANATION

Upon stimulation and stirring the single platelets in whole blood or PRP form aggregates. The aggregation process can be transient and reversible or sustained and irreversible. The extent and reversibility of platelet aggregation depends on the strength and extent of platelet stimulation and is influenced by many other factors including genetic determinants and the presence of drugs that are designed to limit the degree of platelet aggregation. Acquisition of information on the dynamics and extent of platelet aggregation is useful in a wide variety of investigations.

Extensive testing and stability studies have shown that AGGFix effectively fixes platelet aggregates for a period of up to 9 days. The degree of platelet aggregation can be determined by quantifying the change in number of single platelets using a flow cytometer after immunofluorescent staining.

PRINCIPLES OF THE PROCEDURE

At the precise time-point(s) at which quantitation of platelet aggregation in whole blood or PRP is required, an aliquot of the blood or PRP is mixed with AGGFixA in the ratio 1 part to 3 parts of blood or PRP. After 10-30 minutes the sample is diluted with AGGFixB – dilute 1 part of the AGGFixA treated sample with 9 parts of AGGFixB.

The fixed samples may then be transported, stored at room temperature or in a fridge prior to analysis. The suggested procedure to measure platelet aggregation is to quantify the change in the number of single platelets, which can be done using immunofluorescent staining and a flow cytometer. The number of standard beads added to the sample or the number of erythrocytes can be used as a reference for quantitating single platelets. AGGFix has been tested extensively where platelets were identified using CD42a-FITC from Serotec (cat. no. MCA796F).

AGGFix has also been used to quantitate platelet-leucocyte conjugate formation in whole blood. In this case for best results the analyses should be completed within 3 days of fixation. Further information can be provided on request.

REAGENT

The AGGFixA reagent is provided in 5ml bottles and the AGGFixB reagent is provided in 50ml bottles. AGGFixA should be used undiluted and mixed in a 1:3 ratio with the sample to be fixed; subsequently the fixed sample should be diluted 10-fold with AGGFixB. To measure platelet aggregation, typically 15µl of AggFixA is mixed with 45µl of whole blood or PRP at the point at which fixation is required, and 15µl of the treated sample is subsequently diluted with AggFixB up to 150µl. Smaller or larger volumes can also be used.

PRECAUTIONS

1. AGGFix is provided for *in vitro* use only

2. WARNING: AGGFix contains a small quantity of formaldehyde. Formaldehyde is allergenic and exposure can cause cancer. Formaldehyde is harmful by inhalation, in contact with skin and if swallowed. It is irritating to eyes and skin. There is a possible risk of irreversible effects. Formaldehyde may cause sensitisation by skin contact. Consequently keep AGGFix out of the reach of children. Keep the container in a well-ventilated place. Wear suitable protective clothing and gloves. If swallowed, seek medical advice immediately and show this information.
   Dispose of AGGFix according to local regulations for hazardous solutions.

3. All patient specimens and materials with which they come into contact are considered biohazards and should be handled as if capable of transmitting infection.

STORAGE AND HANDLING

The AGGFix solutions are stable for at least 12 months at 4ºC. Do not use either AGGFixA or AGGFixB if the bottle becomes contaminated or precipitation occurs.

SUGGESTED PROCEDURES

Blood Collection
Blood is collected into a suitable anticoagulant; trisodium citrate dihydrate (3.13% w/v, 1ml per 9ml blood) is often used although other suitable anticoagulants such as acid-citrate-dextrose, P-PACK, hirudin or heparin may be used. Tubes are mixed carefully to ensure adequate dispersal of the anticoagulant. Blood may be left at room temperature for a fixed time period (e.g. 10-60 minutes) or processed immediately after venepuncture.

Platelet Activation
Anticoagulated blood or PRP may be stimulated with platelet agonist(s) and the sample is either stirred or shaken at a constant speed and at a defined temperature such as 37°C. All reagents, samples and tubes should be pre-warmed for at least 10 min prior to use if experiments are performed at 37°C.

Following addition of the stimulant, the sample is mixed with a magnetic stirrer bar (e.g. in a standard aggregometer or another stirring device such as the Multi-Sample Agitator [University of Nottingham]), or shaken using a device such as that used with a 96-well plate. The suggested activation time is 4-6 minutes. The sample is then fixed using the two-step procedure described above. After fixation the samples should be mixed well and capped or sealed.

Fixed samples may be stored at room temperature or 4ºC until staining and analysis. If they need to be transported, samples may be placed in a package, appropriately labelled and posted. Samples should be analysed within 9 days following fixation.

LIMITATIONS

Laboratories should establish their own normal reference ranges for their procedures, agonists, patient populations and flow cytometers. Sample stability has been assessed extensively for measurements of platelet aggregation using human whole blood and PRP with the procedures described here.

PERFORMANCE CHARACTERISTICS

Stability studies have been performed using blood from healthy volunteers, patients with acute coronary syndromes and patients with stroke; some of these were treated with aspirin and/or a P2Y12 antagonist. A variety of common platelet agonists used singly and in combination were used for these stability studies. The fixed and stabilised samples may be stored in a fridge or at room temperature for up to 9 days.

Spearman correlations for % platelet aggregation (% fall in number of single platelets) in samples analysed on Day 1 and Day 9 after fixation following storage at room temperature gave R=0.987 ($p<0.0001$). The effects of severe adverse weather conditions during transportation that may cause freezing or result in samples being subjected to higher than average temperatures for extended periods of time has not been fully tested.

TECHNICAL SUPPORT

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