Ristocetin
Ristocetin A for Platelet Aggregation Assays

Product
Ristocetin consists of > 90% Ristocetin A in form of monosulfate.
CAS: 11140-96-1
Formula: C₉₅H₇₂N₄₂O₄₂·H₂SO₄
Mol.-Weight: 2166.01 g/mol (Ristocetinsulfate)
Appearance: white to off-white powder
Solubility: water soluble, clear

Ristocetin is isolated from Nocardia lurida, a Gram-positive bacteria. Ristocetin is an antibiotic, which was withdrawn from pharmaceutical use because of its high toxicity.
Ristocetin has diverse synonyms: e.g. Ristomycin, Spontin, Riston.

Principle
When Ristocetin is added to platelet rich citrate plasma (PRP) it induces in presence of von Willebrand factor platelet agglutination. This can appear as one large wave of aggregation, as well as in two phases. The first wave is induced by von Willebrand factor in presence of Ristocetin and is followed by a second phase which is induced by endogenous ADP (Adenosine diphosphate) released from platelets.

Reagent

Storability
Original closed Ristocetin is stable up to the imprinted expiry date. Use opened flasks immediately.

Risks and Safety
Please maintain the necessary precautions for use of laboratory reagents and body fluids. Applications should be performed by expert personnel only.

For further safety information please see the corresponding Safety Material Data Sheet (MSDS).
Download:
For further safety information please see the corresponding Safety Material Data Sheet (MSDS).
www.msds-id.com/149001-7
or via this QR-Code:

Ristocetin in package sizes delivered by us is not required to be labelled as hazardous/dangerous.

NOT FOR HUMAN MEDICINE OR VETERINARY MEDICAL USE!
ATTENTION! FOR IN-VITRO DIAGNOSTIC USE (IVD)!

Content / Main content
008010-0001
1x 1.0 g Ristocetin Lyophilisate
Also available as OEM-Product (OEM-labeled or as non-labeled bulk ware).

008010-4100
1x 100 mg Ristocetin Lyophilisate
Only available on request as custom-made product.

008010-6105 KIT
10x 15 mg Ristocetin Lyophilisate
Only available on request as custom-made product.

Optional:
008001-0100
1x 100 mL Diluent for Ristocetin

Reconstitution
For determination of von Willebrand co-factor activity a Ristocetin concentration of 10 mg/mL is usually used.

• Warm up Ristocetin and the solvent to room temperature (18 ... 25°C).
• Open vial without loss of substance.
• Add the required quantity of solvent to the vial without loss of substance. Take care about correct pipetting volume and temperature of the solvent (see details of pipette).
• Close the vial.
• Put the vial for about 15...30 min on a roller mixer or another adequate mixer to ensure homogenous mixing.

Working Solution

<table>
<thead>
<tr>
<th>Diluent</th>
<th>Concentration</th>
<th>Sample Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>66.67 mL</td>
<td>6.67 mL</td>
<td>1.00 mg/mL</td>
</tr>
<tr>
<td>71.43 mL</td>
<td>7.14 mL</td>
<td>1.07 mL</td>
</tr>
<tr>
<td>76.92 mL</td>
<td>7.69 mL</td>
<td>1.15 mL</td>
</tr>
<tr>
<td>83.33 mL</td>
<td>8.33 mL</td>
<td>1.25 mL</td>
</tr>
<tr>
<td>90.91 mL</td>
<td>9.09 mL</td>
<td>1.36 mL</td>
</tr>
<tr>
<td>100.0 mL</td>
<td>10.0 mL</td>
<td>1.50 mL</td>
</tr>
</tbody>
</table>

Stability of the working solution
The working solutions can be aliquoted and are stable at 2...8 °C for 7 days. For longer storage aliquots can be frozen at -20 °C for 6 weeks. Mix well after thawing and use within 8 hours.

Recommended equipment and material
Ristocetin can be used with most aggregometers of different manufacturers. Please consult the operating manual of the devices. Additionally required equipment: e.g. aggregometer cuvettes, steel balls, stirrers, pipettes, centrifuge.

Blood collection
Blood collection has to be carried out carefully. Avoid hemolysis and contamination with interstitial fluid. Citrate blood or platelet rich plasma must not be in contact with glass in no case.

Note the time of blood collection.

One-syringe-technique
Blood dilution 9 : 10 (for coagulation), equal to 9 subvolume of blood + 1 subvolume of sodium citrate solution 0.11 mol/L:
• Fill a syringe (5 mL) with 1 subvolume (0.5 mL) of sodium citrate solution 0.11 mol/L.
• Fill 9 subvolumes (4.5 mL) blood into the syringe with the help of a hollow needle wide in diameter. Avoid foam formation and a hailing blood collection.
• Then draw some air into the syringe and mix the content without producing foam.
• Transfer the content of the syringe to a clean disposable plastic centrifuge tube.

Two-syringe-technique
Blood dilution 9 : 10 (for coagulation), equal to 9 subvolume of blood + 1 subvolume of sodium citrate solution 0.11 mol/L:
• Fill into a plastic centrifuge tube 1 subvolume (1.0 mL) of sodium citrate solution 0.11 mol/L.
• Apply lourniquet and puncture vein quickly and clean with a hollow needle wide in diameter.
• Draw ca. 9.5 mL venous blood into a 10 mL disposable syringe, avoid foam formation.
• Reduce blood volume to exactly 9 subvolumes (9.0 mL) and transfer it slowly to the prepared sodium citrate solution in the disposable centrifuge tube and close it.
• Mix sodium citrate solution and blood by careful inversion of the tube.

Preparation of platelet rich plasma (PRP)
• Centrifuge the citrate blood (prepared as described above) for 10 minutes at room temperature at 150 x g.
• If erythrocytes remain in the PRP repeat centrifugation for 5 minutes.
• Transfer the PRP with the help of a plastic pipette to a labeled plastic tube. Close it airtight and let it rest for 30 minutes, so thrombocytes can recover.
• Determine the number of platelets (PLT) in the PRP.
• The PRP-sample is stable for max. 4 hours after blood collection.
Limitations
Remaining erythrocytes in the PRP result in false low aggregation. Thrombocyte numbers below 7500/µl give dubious results. PRP has to rest at least for 30 minutes at room temperature.

Preparation of platelet poor plasma (PPP)
• Centrifuge the remaining blood in the sampling tube for ca. 20 minutes at 1500 x g.
• Transfer the PPP with the help of a plastic pipette to a labeled plastic tube and close it.

Limitations
Thrombocytes in the PPP result in false high aggregation.

Assay procedure (Example)
For performing assays please follow the instructions of the manufacturer of your device.
• Pipette 500 µl (250 µl) platelet poor plasma (PPP) into a cuvette without stir bar and set the aggregometer to blank.
• Into a second cuvette with stir bar pipette 50 µl (225 µl) of platelet rich plasma (PRP) and incubate at 37 °C for 3 minutes.
• Where applicable follow the instructions of the instrument manufacturer to set the baselines 0% and 100%.
• Mix thoroughly Ristocetin working solution prior to every withdrawal and pipette 50 µl (25 µl) directly to the PRP (avoid contact with the tube wall)
• Record aggregation curves at least for 6 minutes.

Reference ranges
Each laboratory should determine its own reference range for Ristocetin aggregation. Usually, Ristocetin with a concentration in the sample of 1.0 mg/mL in normal PRP results in a final aggregation of 55 ... 80 %. An abnormal aggregation may be caused e.g. by von-Willebrand-Faktor or bernard-Soulier-Syndrom, and requires further investigation.

Quality control
Run a control sample like a normal patient sample with each series. For this purpose a sample of a healthy donor, who has not taken medicine the last 10 days, is used.

Indications
Support/Infoservice
Methodical and technical support by eMail (support@bioanalytic.de). In addition you can contact us by phone or fax (German, English).
Check the actuality of this product information periodically on our website

Classification
EU: EDMA: 13 02 04 01 00; IVD

Waste Management
Please see regulations by law of your country.

Literature