EN

Instructions for use

For processing of platelet concentrate (platelet rich plasma, PRP)

1. General Information

Osteokine® is for single use and for handling by trained personnel only. For your own safety wear gloves while handling blood.

Dispose of used material according to regulations for potentially infectious blood products.

- Blood separation-bags contain DEHP. DEHP could influence fertility and may cause damage to the unborn child.
- **Precaution:** Overfilling of the blood separation-bag system (Osteokine®) with more than 60 mL of anticoagulated whole blood may cause rupture or leakage. The disposable product is intended exclusively for single use. Functional faults in the Osteokine® due to incorrect use or defects may cause contamination and spillage of the blood sample.
- **Applications:** The system for the processing of platelet rich plasma is designed for use under aseptic conditions at the point of care for a safe and effective processing of platelet rich plasma (PRP) from a relatively small sample of whole blood (approx. 55 mL).
- ***Carefully read instructions before using the system!***

The following items are required for the processing of autologous platelet concentrate.

Contained within the treatment unit Osteokine® are the following:

1. One Osteokine® (single use) of Rhein Device consisting of:
   - 1 x single-use separation-bag system for processing of platelet concentrates
   - 1 x tube extension for blood transfer
2. Consumables:
   - 1 x 20 G cannula for drawing up the citrate solution (coagulation inhibitor) into the syringe for blood collection
   - 1 x 60 mL syringe for collecting the blood sample
   - 1 x butterfly cannula for collecting the blood sample
   - 1 x 60 mL syringe for drawing up the platelet-poor plasma
   - 1 x 10 mL syringe for drawing up the platelet-rich plasma
   - 2 x 6 mL citrate solution as a coagulation inhibitor (as 10 mL ampoule)
   - 3 x closure caps

The following materials are need but not contained in the treatment unit:

Consumables:
- gloves (if applicable sterile)

Optional (for PRP-gel):
- 5 mL calcium chloride or calcium gluconate 10 %
- if applicable 1000 IU thrombin

Equipment and further materials:
- centrifuge (e.g. Z300, Hermle) with special rotor
- 2 special buckets
- 1 counter balance bottle (plastic, filled with 30 mL water)
- conventional lockable clamp
- waste bin

Optional:
- by using rotors with more than two places: 2 additional buckets for counter balance
- for processing PRP-gel: a small sterile container

For sterile processing two sterile sealed buckets with screw lid and one non-sterile bucket is needed! Consider when planning the usage of Osteokine®.

2. Processing

2.1 Collection of blood sample

Caution: Follow the rules of aseptic blood collection!

- Connect 20 G to cannula to 60 mL syringe and draw up 6 mL citrate solution
- Remove cannula and dispose
- Puncture off vein with butterfly cannula
- Connect 60 mL syringe to cannula tube of the butterfly cannula and **slowly** fill up to the 60 mL mark with blood.
- Immediately after collecting the blood, gently tilt blood-filled syringe 5 to 6 times, to mix anticoagulant (citrate solution) with the blood. **Do not shake!**

2.2 Filling bag system and first centrifugation

- Take Osteokine® out of Tyvek bag. Push slide (**1**) on top of the green disc towards the centre to close the connecting tube (**4**). Remove the **red** closure cap (**2**). **Ensure sterility of the connection** and attach the syringe to the **red** connection (if applicable using the tube extension). **Slowly** transfer the whole content of the syringe into the bag (see fig. 1a + b).

Slide

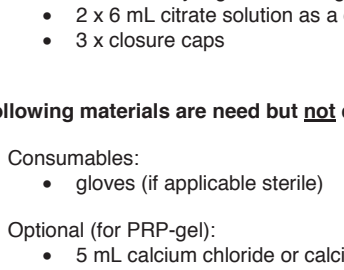

Fig. 1a

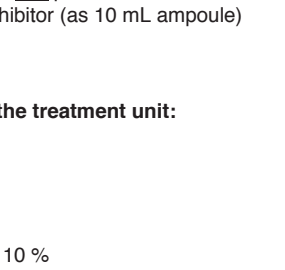
Fig. 1b

- Unscrew the syringe (or tube extension) and close the filling connection (**2**) of the bag with a **new** closure cap.
- Place blood filled Osteokine® (bag system) into one empty bucket (i. a. sterile!). Ensure that the bag plate is correctly aligned in the bucket: The peg on the plastic plate must be inserted into the recess on the inner rim of the bucket (see fig. 1c + d).


Fig. 1c


Fig. 1d

- Close the bucket with **screw lid** (see fig. 1d). Place the closed bucket in the centrifuge (see fig. 1e).


Fig. 1e

Fig. 1f

- Counter balance the bucket (Osteokine®) with the (non sterile) bucket containing the plastic bottle filled with 30 mL water (see fig. 1e + f). Advice: If counter balance is not achieved with 30 mL, adjust the amount of water!
- Close the lid of the centrifuge, set **900 rcf** and **3 min**. Press start.
- At the end of the centrifugation open the lid and **carefully** remove the buckets. **Do not tilt or shake!**

2.3 Separation of red blood cells (RBC) and plasma

- After centrifugation the RBC (**2**) are sedimented at the bottom of bag **A** (see fig. 2). Retract the slide on top of the green disc for opening the connecting tube (see fig. 3). The supernatant plasma (**1**) and the buffy coat (**3**) can now be transferred into bag **B.** Slowly roll bag **A** from the bottom upwards using a conventional lockable clamp. As soon as the connecting tube is completely filled with red elements of blood and the first red cells start to flow into bag **B** (caution: only few red cells should be transferred into bag **B**!), close tube by pushing the slide on the plate.

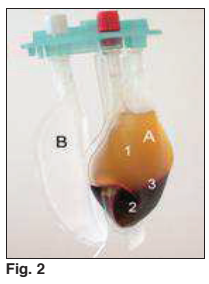

Fig. 2

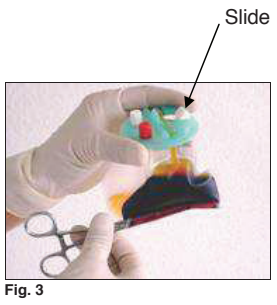

Fig. 3

- Bag **B** now contains the plasma fraction with platelets, white blood cells and a small quantity of RBC. The platelets are uniformly mixed in the plasma and need to be separated by **a second centrifugation** (see fig. 4). Place the bag in the bucket (i. a. 2nd sterile bucket!). Centrifuge at **1500 rcf** for **10 minutes**.


Fig. 4

2.4 Harvesting the end product (PRP)

- After the second centrifugation the bucket containing the bag system is removed carefully from the centrifuge. Through the collection connector (white cap), remove supernatant plasma using the 60 mL syringe. Leave a small residue of 2 - 5 mL (see fig. 5) **Variegate the amount of harvested PRP with the amount of remaining plasma within the bag! With 5 mL residual plasma the amount of PRP is 6 – 8 mL.**


Fig. 5


Fig. 6

- Mix the plasma in bag **B** with the platelet-pellet by **gentle** massage of the bag. Remove PRP from the bag using the 10 mL syringe. The bag can be emptied completely by inverting it while the PRP is being removed (see fig. 6).

Processing PRP-gel (optional):

Transfer the platelet rich plasma from the 10 mL syringe in a sterile container. After adding $CaCL_2$ (or CaGluc, 2 mL 10%), in most cases coagulation process takes 10 to 20 minutes. In order to achieve reproducibility of coagulation 1000 units of thrombin may be added. The addition of thrombin leads to rapid cross-linking of the fibrin, inducing rapid coagulation and better adhesion of the gel to the target tissue. Coagulation is then considerably accelerated (approx. 1 min). The end product obtained is approx. 8 – 10 mL of a platelet rich plasma gel.