

Protocol



Preparing washed blood clots

Version 1.0

Background Various platelet degranulation products are released into serum during blood clotting, including the cytokine TGF-beta. However, it is possible that some fraction of the TGF-beta is retained within the forming blood clot. The purpose of this protocol is to investigate the retention of TGF-beta in the blood clot, which may have pathophysiological significance during normal wound healing and scarring.

Procedure

1. Draw blood using a 21 gauge butterfly needle, without the use of a tourniquet, discarding the first few mls to waste.
2. Dispense in 1ml aliquots into eppendorf tubes.
3. Allow to clot on the bench at room temperature for 2 hours.
4. Spin at 1,000 x g for 4 minutes in a benchtop eppendorf.
5. Remove the supernatant (which may also be retained for assay), and resuspend the clot (with gentle whirlmixing) back to the original blood volume in 150mM phosphate buffer pH 7.0
6. Repeat the spin as above.
7. Repeat steps 5 and 6 a further six times, to give a total of 7 clot washes.
8. After the final spin, resuspend back to the original blood volume in 150mM phosphate buffer pH 7 containing any required additions (e.g. RGDS peptide, gpIIb/IIIa antagonists, plasmin or whatever). Incubate at desired temperature for the desired time-course, sampling the supernatant for assay as required.
9. At the end of the experiment, the amount of TGF-beta remaining in the clot can be determined by achieving complete dissolution of the clot. Spin and remove supernatant, then resuspend by addition of 0.3ml 150mM phosphate buffer pH 2.0 for 30 mins with shaking, followed by neutralisation with NaOH.

KEY POINTS:

* The duration of the clotting time may be crucial. Recent results suggest that platelet degranulation is not complete after 2 hours at room temperature.



Materials

150mM phosphate buffer:

| | | | |
|--------------|---|-------|------------------------------|
| <i>pH7.0</i> | Na ₂ HPO ₄ anhydrous | 6.25g | |
| | NaH ₂ PO ₄ ·2H ₂ O | 4.85g | Make up to 500mL with MilliQ |
| <i>pH2.0</i> | NaH ₂ PO ₄ ·2H ₂ O | 4.85g | |
| | Phosphoric acid [CARE!] | 2.4mL | Make up to 500mL with MilliQ |

Check pH prior to use, and adjust with phosphoric acid / NaOH as required.

Source References

Grainger DJ, Wakefield L, Bethell HW, Farndale RW & Metcalfe JC. Release and activation of platelet latent TGF- β in blood clots during dissolution with plasmin. *Nature Medicine* 1:932-937 (1995)

References where we have used this protocol

DISCLAIMER

Please note that this protocol is provided for information only. While we believe it to be accurate, we do not warrant as to its validity or suitability for any particular purpose. In the event that you wish to use this protocol, we accept no liability for any direct or indirect losses howsoever caused. It is your responsibility to ensure that any use of this protocol is performed safely and in accordance with any local laws, regulations or guidelines applicable to you.