

Camelid PBMCs Isolation Kit (Cat #: FGI-PBMC1930)

Product Description

Camelid Peripheral blood mononuclear cells (PBMC) isolation kit is a ready-to-use kit containing sterile dilution buffers, density mediums and density cushions for isolation of (PBMC) cells from whole blood of various camel family members including Llama, Alpaca.

Principle

Differences in cell density are exploited to separate granulocytes and erythrocytes from peripheral blood mononuclear cells (PBMCs). The most common technique for separating PBMCs is to mix blood with a compound which aggregates the erythrocytes, thereby increasing their sedimentation rate. The active ingredients in the SepOBuffer™ are sodium diatrizoate and polysaccharide with osmolality 290 ± 15 mOsm/L and density of 1.077 ± 0.001 g/ml. The polysaccharide in SepOBuffer™ enhances erythrocyte aggregation increasing erythrocyte sedimentation during centrifugation. PBMCs with lower densities remain in the plasma SepOBuffer™ interface and can be collected from the upper part of the tube when the erythrocytes have settled. The PBMCs from whole blood can be reliably and rapidly isolated in pure form using SepOBuffer™.

Kit Contents:

Description	Cat#	Quantity
 PBMC SepOBuffer (Sodium diatrizoate and Polysaccharides)	FGI-1930	150ml
 PBMC dilution buffer (Isotonic buffer, pH 7.4)	FGI-1931	250ml
 RBC Lysis buffer (Hypotonic buffer, pH 7.4)	FGI-1932	50ml
 PBMC separation tubes with medium grit frit (50ml)	FGI-1933.01	10 Tubes
 PBMC separation tubes with medium grit frit (12ml)	FGI-1933.02	50 Tubes

Procedure

- The high density lymphocyte separation medium, SepOBuffer™ (Cat # FGI-1930) must be brought to room temperature before use.
- Add SepOBuffer™ to sterilized tubes with fused medium grit frit (Catalog # FGI-1933-01).

Recommended Volumes and Tube Sizes

BLOOD (ml)	PBMC dilution buffer (ml)	SepOBuffer (ml)	Tube size (ml)
3	4	4	12
15	15	15	50

- Whole blood is brought to room temperature and is diluted with PBMC dilution buffer (Catalog # FGI-1931).
- Layer the diluted blood in the tube with SepOBuffer™. Care should be taken not to mix diluted blood with SepOBuffer™
- Centrifuge at 850x G for 20 minutes at 25°C in swing out bucket rotor centrifuge without brakes. If the blood has been stored for more than two hours increase the centrifugation time to 30 minutes.
- Discard the upper plasma layer and harvest the buffy coat which contains PBMCs at the plasma-SepOBuffer™ interface.
- The PBMCs in the Buffy coat is pelleted at 1500RPM in swing out bucket rotor centrifuge at 4°C for 10 minutes.
- Wash the cell pellet by re-suspending in PBMC dilution buffer (Cat # FGI-1931) and centrifuge at 1500 RPM in swing out bucket rotor centrifuge at 4°C for 10 minutes.
- Discard the supernatant, Erythrocyte contamination in the PBMC fraction is usually between 1-5%, this can be eliminated by using Lysis buffer (Cat # FGI-1932) as describe below.
- The cell pellet is re-suspended in 5ml of Lysis buffer (FGI-1932) and incubated at room temperature for 5 minutes. After incubation, top off the tube with PBMC dilution buffer (4°C) and centrifuge at 1500 RPM in swing out bucket rotor centrifuge at 4°C for 10 minutes.
- Discard the supernatant and retain the cell pellet which is enriched in PBMCs.
- Cell pellet is re-suspended in PBMC dilution buffer, aliquot into Cryotubes and stored in liquid nitrogen.

Ordering information:

Catalog #	Description	Quantity
FGI-1930	PBMC SepOBuffer (PBMC isolation buffer)	150 ml,300ml
FGI-1931	PBMC dilution buffer	250 ml,500ml
FGI-1932	RBC Lysis buffer	50 ml,100ml
FGI-1933.01	PBMC separation tubes with medium grit frit-50ml	10,20pcs
FGI-1933.02	PBMC separation tubes with medium grit frit-12ml	50,100pcs

Storage

SepOBuffer should be kept sterile and protected from light at 4°C.

* For users who may require bulk quantities of SepOBuffer or PBMC dilution buffer or Lysis buffer can contact us for special discounted pricing.
 This Product is for Research Use Only and is NOT intended for use in humans or clinical diagnosis.