

Cell Treatment with Glass Wool

Purpose:

To remove dead cells, macrophages, etc. which will nonspecifically bind reagents

Materials:

Glass wool may be used as is or washed with chromic acid and rinsed overnight in tap H₂O.

When thoroughly washed, dry in oven

5 ml plastic syringe (adequate for 1-2 spleens)

Medium (with or without FCS)

Spleen cells prepared as usual (leave at room temp while preparing column)
or resuspend in warm buffer

Method:

Gently pack syringe ¼ full with dry glass wool.

Pass through ~30 ml of medium to stabilize pH of column – flow rate of fast drip

Swirl column between hands to warm slightly

Pass cells slowly into glass wool and allow to stand 1 min at room temp

Wash with 37°C medium until clear (10-15 ml is adequate)

DO NOT ALLOW HEAD OF BUFFER PBS TO FORM OVER COLUMN

Centrifuge.

Wash column with warm 37°C buffer and elute column c warm buffer

Or at least 1ml of glass in syringe/spleen expect to lose ~ 30-50% of cell # on glass

Do not let cells sit on glass. Just apply cells and wash off.