USE OF SLIDING MICROTOME (prepared by Beth Friedman) (SOP-64)

- Remove oil from knife with 100% ETOH: Use Kim Wipes to clean fluid from top surface of knife, taking care not to slide Kim Wipes into the edge of the knife, which will dull it

- Set up vessels for tissue collection including-
    - Crystallization dish with buffer for section preview
    - TC multi-well plates
    - Paint brush
    - Black paper
    - Single edge blade for trimming and blocking
    - Large forceps for handling tissue
    - Squeeze bottle filled with buffer
    - Kim Wipes
- Label TC plates with
    - DATE ,
    - RAT #,
    - SECTION THICKNESS,
    - # OF PLATES ANIMAL
    - SERIES (e.g., 1 section / well in 24 successive wells = 1/24)
- FILL WELLS WITH PBS CONTAINING 0.1% (w/v) SODIUM AZIDE
- Block tissue so it is ready to place on stage - area of interest should be in center of block
- Turn Cooling Water On
- Turn on cold stage (uncover microtome first)
- Check sensor is in object stage position
- Chill cold stage down to about -30
- Lower cold stage so knife won't hit it
- Place cleaned knife in the holder - carefully. Then tighten the screws and check the angle.
- Attach block (blot excess fluid) to cold stage with thin film of buffer.
- Let block freeze (about 5 minutes).
- Equilibrate block to warmer temp. – about –24^{o} C.
- Check section thickness knob.
- Level surface block with stage adjustments- tighten stage adjustment lever.
- Raise stage to let block almost meet knife edge.
- Face off block.
- Collect sections with moist (not dripping) brush and transfer to buffer.