Prepared by: Steve Brown	**SOP – TISSUE CULTURE WORKUP**
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PURPOSE:
To establish a standard operating procedure for tissue culture infection and measurement of tau.

PROCEDURE:

VIRUS PREPARATION

1. Split 293T cells to 10cm dishes so that they are semi-confluent the day of transfection.
   1.1. Usually a confluent plate split 1:6 the day before transfection is about correct.
   1.2. Plates should contain 8ml DMEM/10%FCS/1%pennicillin-streptomycin-glutamine
2. Make DNA mix – plasmids, 100ul CaCl2 SOLUTION, H2O to 500ul.
   2.1. CaCl2 SOLUTION – 2M CaCl2, tissue culture grade, filter-sterilized.
   2.2. Plasmids – 14ug coding plasmid, 10ug p8.91 packaging plasmid, 6ug pMD2G coat plasmid.
      2.2.1. Plasmids should be of high purity: Quiagen maxi-prep, JetStar maxi-prep, or CsCl2-purified, dissolved in H2O.
   2.3. Water used for transfection should be buffered with 2mM HEPES pH 7.05.
3. In 4-10ml polypropylene tube, place 0.5ml 2xHeBS.
   3.1. 2xHeBS – 6mM dextrose, 137mM NaCl, 5mM KCl, 0.75mM Na2HPO4*7H2O, 25mM HEPES (free acid). Then ajust to pH 7.05 with NaOH
   3.2. pH of HeBS is absolutely critical. 7.05 is ideal, less than 7.0 or more than 7.1 is useless.
4. Add DNA to HeBS solution in fashion specified.
   4.1. All solutions should be at room temperature.
   4.2. Bubble air into HeBS with pipet. Add DNA dropwise with pipet tip, letting it slide down side of bubbling pipet.
   4.3. Vortex 10 seconds.
   4.4. Incubate exactly 30 minutes at room temperature.
5. A fine white translucent precipitate should form. Add dropwise to medium.
   5.1. Separate mix per plate; do not do in batch.
   5.2. Mix precipitate and medium by rocking plate back and forth, as for spreading cells.
6. After 24 hours of incubation in standard incubator, change medium.
   6.1. Rinse cells 1x with PBS.
   6.2. Replace medium with 10ml fresh medium.
7. After 24 hours, harvest medium containing virus, and replace with 10ml fresh medium.
   7.1. Store viral supernatant on ice.
8. After another 24 hours, harvest supernatant again and pool with first medium.
   8.1. Plates can now be discarded.
9. Spin supernatant 2k 5 minutes to pellet debris.
10. Filter through 0.2-0.4micron filters. Store in aliquots at -80.
11. Supernatant can be concentrated by ultracentrifugation, 30,000 rpm 90 minutes in mini-ultracentrifuge or comparable.
12. Resuspend overnight in desired volume of medium by medium-speed vortexing in automatic vortexer.
    12.1. 10x concentration is ideal. More than this is toxic.

INFECTION AND MEASUREMENT

1. Thaw primary fibroblasts.
    1.1. Unfreeze quickly in 37 degree waterbath, and dilute into 10ml PBS.
    1.2. Spin 1000 rpm 5 minutes, and aspirate supernatant.
    1.3. Resuspend in 2ml growth medium, and plate to 4 cm. dish, if appropriate.
2. The day before infection, split confluent cells 1:4 to 2x4cm dishes.
3. The day of transfection, remove medium and add warmed viral supernatant.
    3.1. Don't forget to increase serum to 20% if appropriate.
    3.2. For immortalized cells, addition of polybrene to 1ug/ml final concentration is often helpful. To primary cells, rather add 8ug/ml protamine sulfate final concentration.
4. Change to fresh supernatant after 6-24 hours, and let cells recover overnight.
5. Repeat 1x-2x as desired. If cells become confluent during this time, split 1:2 again as necessary.
6. Rinse cells, and replace medium with fresh growth medium.
7. After 3 days, conduct measurement.
    7.1. Treat cells 15 minutes with INDUCTION MEDIUM.
        7.1.1. INDUCTION MEDIUM – growth medium + 100nM dexamethasone (from 1/10,000 stock in EtOH)
    7.2. Rinse cells 2x with 1ml PBS.
    7.3. Replace medium with 3ml COUNTING MEDIUM.
        7.3.1. COUNTING MEDIUM – DMEM high-glucose without phenol red, 20% FCS, 1% pen-strep-glu, 0.1mM luciferin (from 1000x stock in H2O).
    7.4. Place cells into machine.
8. Count in 5-minute bins for 5 days or until rhythms dampen to flatness.