

Click's Medium

On day of use:

1. ~96 mls Click's medium (Irvine Scientific #9195) stored in cold room
2. 1 ml HEPES 100X (final 4.57 mM) stored in cold room
3. 2 mls 50X "P" (penicillin, streptomycin, glutamine, gentamicin, + NaHCO_3) stored frozen in 2 ml and 4 ml aliquots in -20°C freezer near PCM office
4. 100 μl 1000X 2-ME; stored in cold room; final conc = 50 μM
5. Antigen
6. 0.5 ml- 1.0 ml Normal mouse serum(final 0.5%- 1.0%): Use serum from freshly collected blood (allow blood to stand at room temp for 10 minutes; followed by incubation on ice for 30 minutes; spin 2000 rpm, 5 minutes). OR use frozen serum which has been stored at -70°C and contains 2-ME at a final conc of 50 μM .

Remember to re-filter the entire mix if the serum was collected in a non-sterile tube.

1000x 2-ME:

34.2 μl of 14.2 M stock up in 10 mls BSS.

1 M Hepes pH7.4:

Make 100 mls 1 M NaHepes (26 gm/100mls) and
100 mls 1M Hepes (23.83 gm/100mls)

Mix 42 mls NaHepes + 58 mls Hepes.

Test pH.

Sterile filter.

<u>50X Pen-Strep:</u>	<u>100 mls</u>	<u>200 mls</u>
Penicillin G	300 mg	600 mg
Streptomycin Sulfate	500 mg	1 gm
Glutamine	2.92 gm	5.84 gm
Gentamycin Sulfate	250 mg	500 mg
NaHCO_3	4.215 gm	8.63 gm
Up to volume in dH_2O		