Evaluation of Intracerebral lesions
GABA-transaminase Method

Ingredients
KCl 1.6 mM (FW 74.55)
MgSO4 1.2 mM (FW 120.4)
Tris HCl (pH 7.4) 100 mM (FW 152.6, Sigma T5128)
Nitro-blue tetrazolium 2.4 mM (FW 817.6, Sigma N6876)
DMSO (Sigma D5879)
b-NAD 3 mM (FW 663.4, Sigma N-7004)
a-ketoglutarate 5 mM (FW 184.2, [Anhydrous] Sigma K2000)
GABA 50 mM (FW 103..1, Sigma A2129)

To prepare 100 ml of the incubation mixture
1- Nitro-blue 200 mg in 100 ml ddH2O (2 mg/ml) + 0.5 ml of DMSO. Agitate
2- Dissolve Tris-HCl 6.28 g in 19 ml dd H2O. Adjust pH to 7.4 with NaOH 10 N and fill to 20 ml with dd H2O.
3- Add 5 ml (1.57 g) of Tris-HCl to Nitro-blue while agitating.
4- Add 500 mg of a -ketoglutarate (5 mg/ml) to 25 ml of ddH2O (slowly, tends to make the solution precipitate)
5- Add 14 mg Mg SO4 (0.14 mg/ml) (add more if hydrated form)
6- Add 520 mg of GABA (5 mg/ml)
7- Add 200 mg of NAD (2 mg/ml) and keep solution on ice.
8- Adjust final pH to 7.4. To prepare tissue
The animal is deeply anesthetized and the whole brain is quickly removed and frozen on powdered dry ice. The brain is the put into a plastic tube on dry ice, sealed, and stored in the -80oC freezer until ready to section.
Sections (12-14 um) are cut in a cryostat and immediately mounted on cold slides which are briefly brought to room temperature to allow sections to adhere, and then put into a dessicated chamber (preferably under vacuum) at -20 to 80oC for a minimum of three hours to preserve enzymatic activity. Critical factors in preserving enzymatic activity pH: If the pH < 6.5 no reaction is seen (I don't know yet about a pH above 8). Optimal pH is 7.4. To: GABA-T is very sensitive to room temperature, especially when the sections are still wet. After sections have been melted on to glass slides the have to be fully dried at freezing temperatures before being exposed to the incubation medium.

To react tissue
1- Remove sections from cold chamber and briefly put them on 40oC plate to prevent condensation (the enzyme is water soluble)
2- Create a wall around section using PAP pen.
3- Drop 100-200 ul of solution on each section and incubate for 30-45 min at room temp or 40oC in humid chamber.

4- Wash slide twice in Tris buffer
5- Fix in 10% formalin (pH 7.4) for 1 hour
6- Wash slides twice in Tris buffer
7- Dehydrate in methanol 70% x 5 min; 90% x 5 min; 95% x 5 min; 100% x 5 min (twice)
8- Defat in xylene x 5 min twice and coverslip.

The reaction product should be dark purple.

Gale et al. (1984) Brain Res 307:255-262

Should be 100 ml to obtained the concentrations suggested in Gale et al (1984). We found, however, that a precipetate forms after adding the Alpha-ketoglutarate in a 100 ml volume. Because much less cloudiness of the solution is seen with 150 ml and the reaction works well, we use this higher dilution volume.