**Oxygen Glucose Deprivation of Primary Neurons**

**Preparation:**

* Use primary forebrain neurons at DIV20-25 grown on glass coverslips
	+ At this age, the neurons express mature NMDA receptors essential for proper responses to OGD
	+ Glass coverslips are used as they can be transferred into 35mm dishes that have been shown to bind and retain significantly less oxygen than other types of plates
* Prepare appropriate amounts of fresh MEM/BSA/Hepes (wash) media
* Prepare appropriate amounts of fresh MEM/BSA/Hepes/N2 (recovery) media
* Prepare necessary amounts of OGD media
* Warm wash media, recovery media and OGD media in 37oC waterbath
* UV the hypoxic chamber (Billups-Rothenberg) for 20’
* Label 35mm dishes appropriately

**Experimental Procedure:**

* De-gas the pre-warmed OGD media for 5’ by bubbling with a gas mixture composed of 10%H2/85%N2/5%CO2
	+ This can be done by placing a 2mL pipette into the OGD bottle with N2 flowing through it at 5psi.
* Place 2mL of de-gassed OGD media into the 35mm dishes
* Transfer glass coverslips to the appropriate 35mm dishes containing OGD media
* Aspirate the initial 2mL of OGD media (to wash away any remaining media) and add 2mL of de-gassed OGD media
* De-gas the entire hypoxic chamber:
	+ Place the dishes containing the coverslips in 2mL OGD media (lids off) inside the hypoxic chamber
	+ Connect the gas mixture to the entrance valve and leave the exit valve open
	+ Flush the chamber with the gas mixture (5psi) for 5’
* Following the 5’ flush:
	+ Close the clamp on the exit valve tubing first and then close the clamp on the entrance valve tubing.
* Place the entire chamber into a 37oC incubator for the desired OGD length.
* Once the OGD time is complete, return the chamber to the hood and aspirate off the OGD media
* Add 2mL of wash media to each dish
* Aspirate the wash media and replace with 2mL of recovery media
* Place the lids back on the dishes and return to the incubator for the desired recovery time.

**Medias:**

* **OGD Media (Glucose free Salt Solution)**
	+ 150mM NaCl
	+ 2.8mM KCl
	+ 1mM CaCl2
	+ 10mM HEPES
	+ Water
		- pH 7.4
* **Wash Media (MEM/BSA/HEPES)**
	+ MEM
	+ 0.01% BSA
	+ 25mM HEPES
* **Recovery Media (MEM/BSA/HEPES/N2)**
	+ MEM
	+ 0.01% BSA
	+ 25mM HEPES
	+ 2X N2 supplement

**Ordering Information:**

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| **Product** | **Company** | **Catalog #** |
| MEM | Invitrogen | 51200-038 |
| HEPES | Sigma | H0887 |
| N2 | Invitrogen | 17502-048 |