



Primary Culture Preparation with Wheaton Celstir® Cell Culture Flask

Abstract

Wheaton Science Products Celstir® Cell Culture Flasks are most commonly used for growing cells in culture, but are widely used to prepare cells, especially neurons, for other cell preparation. Celstir® flasks are useful for preparing cells because they can be closed to maintain sterility of the culture. Jacketed flasks can also be used to maintain the optimal temperature for the cells, saving incubator space and reducing the disturbance to other cultures while preparing primary cultures.

Background

A wide number of researchers have used the Wheaton Celstirs® to prepare cells for other cultures. Generally, the spinner flask is used to gently agitate the cells in culture medium with a trypsinizing agent added. The cells are often prepared with Jacketed Celstir® flasks because of the ease in maintaining optimal conditions for the cells by regulating the temperature of the water flowing through the jacket. Typically, 25-50 mL flasks are used for cell preparation.

Procedures

Neurons

Neurons are adherent cells that do not generally grow in suspension culture. Neurons are generally cultured for a short period of time on slide cover slips or small dishes, where they are manipulated and observed.

Neurons are generally obtained from primary tissue and dissociated to individual cells using a combination of chemical and mechanical techniques.

For example, Martin¹ et al placed brain slices from male Sprague-Dawley rats into a Jacketed Celstir® flask containing HEPES buffer, papain, NaCl, KH_2PO_4 , glucose, glutathione, nitro-arginine, kynurenic acid, and pyruvic acid. The cells were digested at 36°C by circulating water in the jacket of the flask. The cells were rinsed, and further triturated with Pasteur pipettes with successively smaller tip diameters. This is similar to the technique used by D. James Surmeier². The cells were dissociated in HEPES buffered Hank's Balanced Salt Solution. After digestion, the cells were triturated with graded Pasteur pipettes.

Myocytes

Haneda³ et al dissociated myocytes in a Wheaton water jacketed Celstir® in a solution containing trypsin, chymotrypsin, and elastase in HEPES buffered Hank's salt solution at 37°C. The tissue was minced prior to dissociation in the flask.

Decidual Tissue

Tessier⁴ et al started primary decidual cell culture from tissue obtained from pseudo-pregnant rats. The tissue was incubated in a water-jacketed Celstir® flask at 37°C in RPMI 1640 medium supplemented with collagenase and deoxyribonuclease.

Conclusion

Wheaton Celstir® flasks are an excellent alternative to trypsinizing flasks for small volumes of cell culture. They can be sealed against contamination with the caps that are provided with the flasks, and stirring is done with a magnetic stirrer. The flasks may be placed in an incubator, or jacketed flasks can be used to maintain the cells at optimal temperature. Use of jacketed flasks reduces the demand for incubator space and eliminates disturbance of other cell cultures from the continual opening and closing of the incubator during primary cell culture preparation. Wheaton Celstir® flasks can be used for a variety of cells and the cultures can be grown in larger Celstir® flasks after the cells are dissociated.

¹ Martin, G.; Fabre, V.; Siggins, G.R.; de Lecea, L. Interaction of the hypocretins with neurotransmitters in the nucleus accumbens. *Reg. Peptides* 104 111-117, 2002

² Yan, Z.; Surmeier, D.J. Dopamine Receptors Enhance Zn^{2+} -Sensitive GABA_A Currents in Striatal Cholinergic Interneurons through a PKA/PP1 Cascade. *Neuron* 19 1115-1126, 1997

³ Oi, S.; Haneda, T.; Osaki, J.; Kashiwagi, Y.; Nakamura, Y.; Kawabe, J.; Kikuchi, K. Lovastatin prevents angiotensin II-induced cardiac hypertrophy in cultured neonatal rat heart cells. *Eur. J. Pharmacology* 376 139-148, 1999

⁴ Prigent-Tessier, A.; Tessier, C.; Hirokawa-Takamori, M.; Boyer, C.; Ferguson-Gottschall, S.; Gibori, G. Rat Decidual Prolactin – Identification, Molecular Cloning, and Characterization, *Journal Bio Chem*, Vol 274, 37982-37989, 1999