absence of any metals.

E333

Purification of the *E.coli* expressed p60 Protein from Listeria welshimerill by Amylose Resin Based Affinity Chromatography

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The Listeria welshimerill is an animal and human pathogen and its p60 protein is a major extracellular protein, which is encoded in iap(invasion associated protein) gene. These proteins are believed to be involved in the invasion of these bacteria into their host cells. To produce p60 in *E.coli*, the iap gene was recombinantly cloned and overexpressed. A purification protocol was developed for MBP(maltose binding protein)-p60 fusion protein by amylose-resin based affinity chromatograpy. The purified MBP-p60 was detected either as denaturated or neutralized form using a specific p60 monoclonal antibody. The method might be an easy alternative to common purification protocols of p60 from Listeria spp. for antibody production.

E334

Studies on Mechanism and Binding Proteins of Human HtrA2, Serine Protease

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Human HtrA 2 (omi) is a recently described novel member of the mammalian serine proteases family homologous to the Escherichia coli Htr A gene that are essential for bacterial survival at high temperatures. Although the physiological function of this new family is unclear, the current understanding is that as well as being involved in the degradation of aberrantly folded proteins during the conditions of cellular stress, they may posses a chaperon-like role under nomal conditions. For understanding the mechanism of the HtrA 2 protein, we isolated HtrA 2 binding protein using the yeast two-hybrid assay. The pLex-HtrA 2, containging the gene for N-terminal region of the HtrA 2 inserted into PEG202 vector, was used as a bait plasmid to identify interacting protein gene from a human HeLa cDNA library. Final 16 X-gal positive colonies were selected from 1×107 colonies, isolated the plasmid containing the library cDNA and sequenced. The selected sequence represented the human karyopherin alpha 2, human embryonic ectoderm development protein (HEED), NADH dehydrogenase (ubiquinone) 1.

E335

HIV-1 Nef Decreased the Transcription Activity of the CREB-2

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Nef is a 27kDa myristoylated phosphoprotein expressed early in infection by HIV-1. To find the HIV-1 Nef interacting protein from T Jurkat cell, yeast two hybrid system was performed. The sequence determination and homology search of the isolated cDNA showed that one of the selected colonies encode the human cAMP

responsive element regulatory protein, CREB-2. Previously, we showed specific interaction between the Nef and CREB-2 by using the in vitro protein binding assay. We showed that the basic leucine zipper carboxy terminal domain of CREB-2 is required for the interaciton with the HIV-1 Nef by using in liquid β-galactosidase assay. And, we demonstrated that CREB-2 cooperates with Nef to enhance viral transcription. Here, we report the construction of the CRE-SEAP reporter plasmid and the stable cell line in Jurkat cell. Using this plasmid investigated the activity of the transfected cells. CREB-2 has the effect on the transcription activity on the pGL2 promoter when we performed the transient transfection assay with Nef and CRE.

E501

Alteration of Glutathione-Ascorbate Cycle in Tomato Seedlings Exposed to Cadmium

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Developmental changes of glutathione-ascorbate cycle in seedlings of tomato (*Lycopersicon esculentum*) exposed to toxic cadmium concentrations (up to 100 mM for 9 days) were investigated. The contents of total protein and thiols in both leaves and roots and total glutathione in leaves increased but the content of total ascorbic acid in leaves was maintained unchanged with cadmium exposure. Further, the ratio of oxidized glutathione (GSSG)/reduced glutathione (GSH) in both leaves and roots increased but the ratio of dehydroascorbate/ascorbate and the activity of glutathione reductase in leaves decreased with cadmium exposure. Our results imply that tomato seedlings exposed to toxic levels of cadmium experience oxidative stress which might be caused by the impaired glutathione-ascorbate cycle resulting from the altered activities of enzymes and the lowered production of antioxidant compounds involved in the oxidation-reduction cycle.

E801

Development of the Screening System for Antagonist of PPAR-γ by Using Yeast Two Hybrid System

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Obesity, an excessive accumulation of adipose tissue, is characterized by an increase in the number or size of adipocyte, or both. Adipocyte is highly specialized cell that play a critical role in energy homeostasis. Adipocyte differentiation is characterized by a coordinate increase in adipocyte-specific gene expression. Recently PPAR-γ was shown to be expressed in an adipose-specific manner and its expression induced early during the course of differentiation of several preadipocyte cell line. PPAR-γ and the retinoid X receptor α (RXRα) form a heteromeric complex that function as a central regulator of adipocyte differentiation. We developed the screening method to search the candidates for the inhibitors of the differentiation of preadipocyte to adipocyte, by using the yeast two hybrid system. The bait and prey were RXR and PPAR-γ, respectively. Using this system, we found several candidates from the culture broth of Streptomyces sp. and some plant extract.

E801

Oxygen Defense Role of Xanthine Dehydrogenase