

Supplemental Material for Environmental Health Perspectives

Global Levels of Histone Modifications in Peripheral Blood Mononuclear Cells of Subjects with Exposure to Nickel

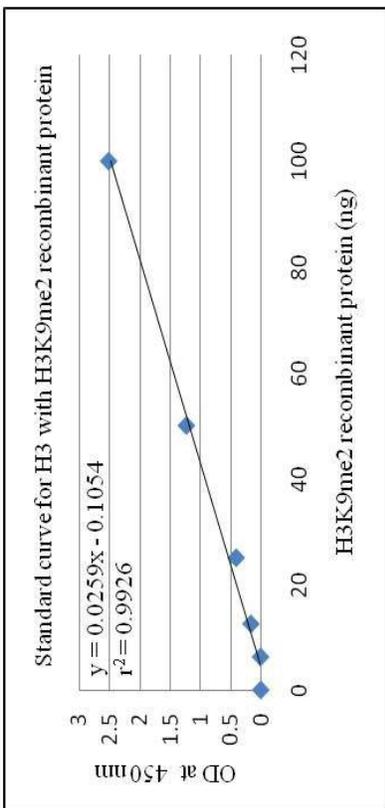
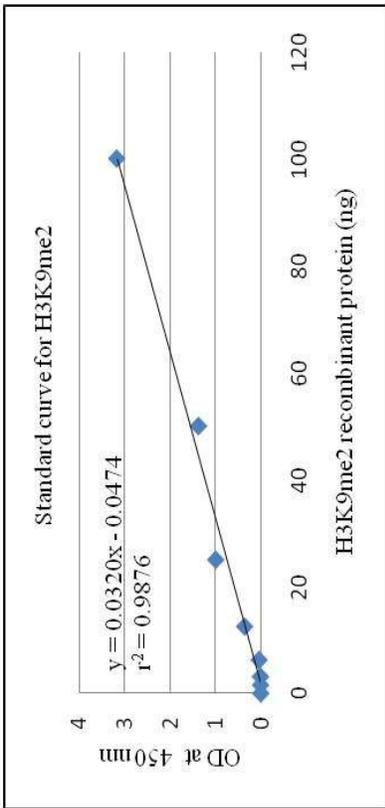
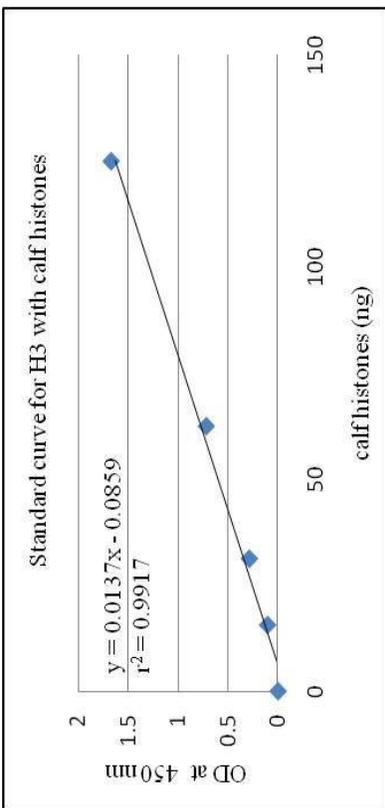
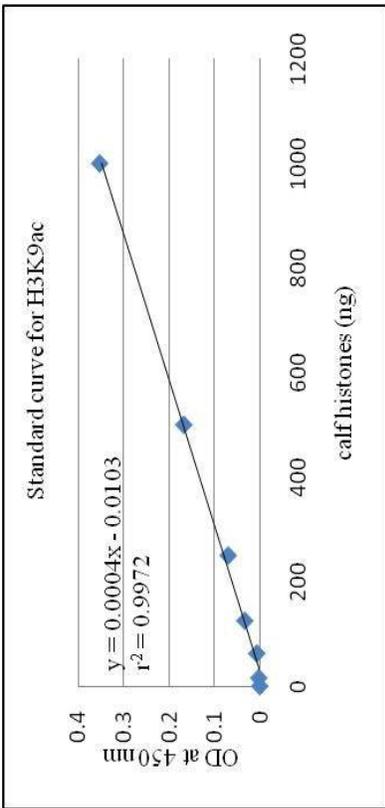
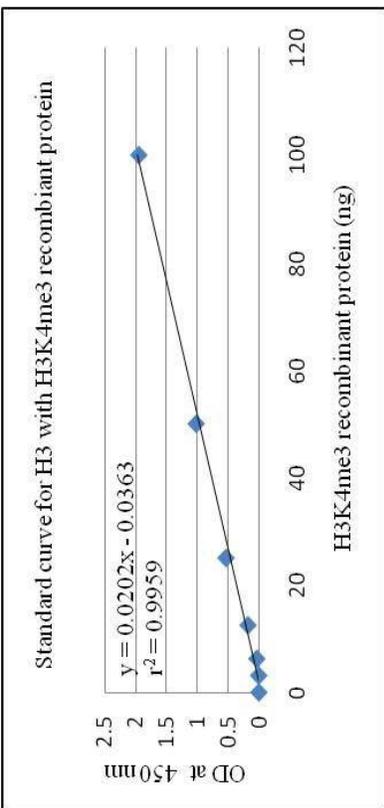
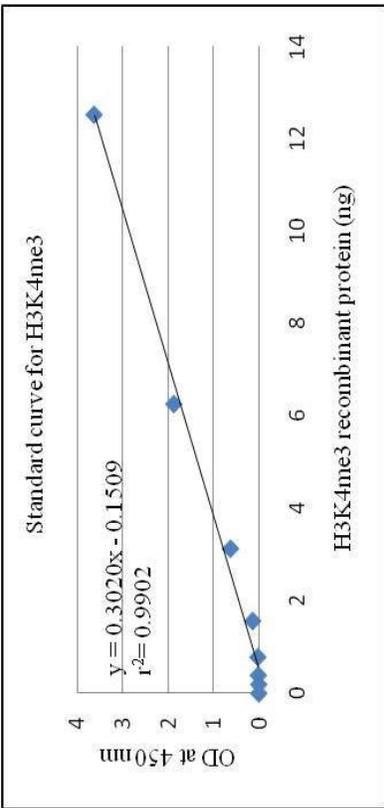
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Supplemental Material, Figure 1. Standard curves for measurement of global levels of H3K4me3, H3K9ac, and H3K9me2 histone modifications with enzyme-linked immunoabsorbent (ELISA) assay. Standard curves for H3K4me3 and H3K9me2 were calculated with H3K4me3 or H3K9me2 recombinant proteins (Active Motif, Carlsbad, CA). Recombinant *Xenopus Laevis* proteins are produced in *E.coli* and purified using Fast protein liquid chromatography (FPLC). The purified proteins are substantially alkylated to specifically trimethylate H3K4me3 or dimethylate H3K9 and repurified prior to lyophilization. The degree of methylation is >99% for H3K4me3 and H3K9me2 recombinant protein. Samples for standard curves for H3K9ac were prepared with calf histone proteins (Sigma, Saint Louis, MO). Standard curves for H3K4me3 were prepared with serial dilutions beginning with 12.5 ng of the recombinant protein. Standard curves for H3K9me3 and H3K9ac were prepared with serial dilutions beginning with 100 ng of recombinant protein or 1000 ng calf histone proteins, respectively. To calculate histone loading, standard curves for H3 were also prepared with the H3K4me3 or H3K9me2 recombinant proteins or calf histones. Standard curves for H3 for H3K4me3 or H3K9me2 were prepared with serial dilutions beginning with 100 ng H3K4me3 or H3K9me2 recombinant protein. Standard curves for H3 for H3K9ac were prepared with serial dilutions beginning with 125 ng calf histones. All standard curves were performed with concentrations up to where the conditions were linear.