Introduction
Lubiprostone is used clinically to treat chronic idiopathic constipation and irritable bowel syndrome with constipation. It is a prostone (derived from metabolites of prostaglandins) that activates CIC-2 Cl channels with an EC₅₀ of 20 nM and increases intracellular chloride secretion. In the present study, the agonist and antagonist activity of lubiprostone on cloned human EP and FP receptors was examined to more directly determine the EC₅₀ for lubiprostone binding to these specific receptors. This reduces the need to use either EP receptor antagonists or complex biological processes such as contraction to infer binding of lubiprostone to prostaglandin receptors.

Methods
Lubiprostone binding to recombinant EP₁, EP₂, EP₃, EP₄ and FP receptors was assayed by Millipore Corporation, Bioscience Division (St. Charles, MO), using Chemiscreen calcium optimized FFLIR cell lines containing high levels of the procuglanic protein, Galphal₁α₁, to enhance coupling of the receptor to the calcium signaling pathway. These cells were transfected with cDNA containing either full-length human EP₁, EP₂, splice variant 6 of EP₂, EP₃, or EP₄ receptors. Triplicate assays of lubiprostone effects were carried out with receptors expressed in cultured cells. Data represent the mean (± SE) of 3 determinations.

Figure 1. Agonist Effect of Lubiprostone on Cloned EP and FP Receptors

Results

Figure 1. Dose response curve for agonist activity of lubiprostone on cloned EP₁, EP₂, EP₃, EP₄ and FP receptors expressed in cultured cells. Data represent the mean (± SE) of 3 determinations.

Figure 2. Dose response curve for antagonist activity of lubiprostone on cloned EP₁, EP₂, EP₃, EP₄ and FP receptors expressed in cultured cells. Data represent the mean (± SE) of 3 determinations.

Figure 2. Antagonist Effect of Lubiprostone on Cloned EP and FP Receptors

Summary
It has been shown in the present study that lubiprostone does not act as an agonist on cloned human EP₁, EP₂, or FP receptors. The lack of agonist activity of lubiprostone on cloned human EP₁ contradicts the finding that lubiprostone reduced electrically stimulated neural contractions in rat and human colon circular muscle with an EC₅₀ at near nanomolar levels that were inhibited by an EP₂ (but not other EP) receptor antagonist and implies that these reported effects are not due to EP₂ receptor occupancy by lubiprostone.

Agonist activity of lubiprostone on cloned EP₁ was very low with an EC₅₀ = 330 nM, a value 44 times higher than for PGE₂ on the EP₁ receptor. Moreover, this concentration is approximately 15 times higher than the EC₅₀ for lubiprostone for activation of ClC-2 chloride channels. Lubiprostone remains mostly in the lumen of the gut and does not enter the circulation. This means that lubiprostone will not reach sufficiently high concentrations to activate EP receptors in the stomach muscle layer. Since EP₂ antagonists do not affect the PGE₂ effects on the vagal nerve, lubiprostone is unlikely to act on the stomach through EP₂ (or other EP or FP) receptors causing vagal nerve stimulation. This study confirms the previous findings of Ueno et al. that demonstrated that lubiprostone does not have pharmacologically relevant activity on prostaglandin receptors.

Conclusions
At clinically relevant doses lubiprostone is unlikely to have significant PG receptor activity. The results demonstrate that activation of CIC-2 chloride channels underlying the clinical effects of lubiprostone is independent of EP₁ or FP₂ receptor occupation.

References