TRAIL (TNF-related apoptosis inducing ligand) became known for its ability to selectively eliminate cancer cells. This ligand is a member of the TNF (tumor necrosis factor) ligands family and triggers extrinsic apoptotic pathway by binding of its death receptor 4 or 5 (DR4/5), and subsequent formation of death-inducing signalling complex (DISC). This signalling complex is required for successful transmission of apoptotic signal and activation of proximal caspases. However, regulation of the initial steps leading to activation of caspases is still not fully understood. Endocytosis of a TRAIL- DR4/5-DISC complex can be one of modulators of the initiation of extrinsic apoptotic pathway. Recent studies show controversial data documenting that endocytosis of TRAIL receptosomes can in cell type specific manner either positively or negatively influence TRAIL-induced apoptotic signalling. In this study, we focus on the analysis of a role of endocytosis and acidification of endosomal compartments during TRAIL-induced apoptosis in human colorectal cancer cell lines. Our results support the view that both clathrin-dependent endocytosis of TRAIL receptosome and endosomal acidification positively affect activation of caspases during the early stages of TRAIL-induced apoptosis. Inhibition of endocytosis or endosomal acidification causes significant and reproducible drop of apoptotic signalling in first hour after TRAIL treatment. However, this inhibitory effect is only temporary and it does not lead to full-blown inhibition of apoptosis. Suppression of acidification by V-ATPase inhibitors causes an overall slowdown of apoptotic signaling and disruption of the endosomal trafficking of TRAIL receptosomes. This study shows that endocytosis and endosomal acidification of TRAIL receptosomes have positive effect on apoptotic signaling and accelerate the early TRAIL-induced apoptotic events in tested colorectal cell lines.