Mixed chitosan and calcium alginate beads were prepared for its possible use in the protection of specimens for cryopreservation with improved functionality. An additional layer of chitosan was added to preformed calcium alginate beads. Chitosan is a relatively inexpensive and widely available polysaccharide, obtained as a food byproduct, with interesting properties in alimentary, agricultural, biomedical and chemical fields, and biocompatible, nontoxic and biodegradable properties. The antimicrobial character of this polymer has proved to be of special utility, protecting beads against bacterial and fungal proliferation, while chitosan antioxidant properties could have a protective roll on cryopreserved specimens against oxidative damage. Chitosan of several molecular weights were studied and tested. The complementary charges of alginate and chitosan polymers favored the interaction and the formation of the external chitosan layer. This interaction between polymers was characterized by Fourier transfer infrared spectroscopy (FTIR), showing how calcium ions interacting with alginate were partially replaced by chitosan. Information on the internal and external mix-bead architecture was obtained by microscopic techniques, such as scanning electron microscopy (SEM) and low-temperature scanning electron microscopy (cryo-SEM). A smooth, crack-free surface was observed. The external additional chitosan layer have only a small influence in the more relevant physical parameters for mixed beads employment for cryopreservation purposes, such as water and solute intake and loss in dehydration. Consequently, the additional chitosan layer does not appear to significantly alter beads water permeation behavior. Additionally, freezing thermodynamic parameters appear also only slightly altered, after differential scanning calorimetry (DSC). The general conclusion is that chitosan-coated calcium alginate beads behave in a similar way to the uncoated ones for most cryopreservation purposes. Consequently, chitosan addition can be used to protect beads used in encapsulation procedures against microbial contamination and oxidative damage, other properties not being significantly altered.