In vitro Motility Assay

The *in vitro* motility assay was carried out according to published protocols (*92-95*). The flow-through microchamber used had an internal volume of ~10 µl. First, 15 µl of the myosin construct (1.5 mg/ml) in Assay Buffer (AB, 25 mM imidazole-HCl, 25 mM KCl, 4 mM MgCl2, 1 mM EGTA, 1 mM DTT, pH 7.4) was pipetted through the microchamber and incubated at room temperature for 1 minute. Nonspecific binding sites were blocked by washing in 100 µl 0.5 mg/ml BSA in AB buffer. Actin filaments were added at a concentration of 70 ng/ml and allowed to bind to the myosin-coated surface for 1 minute. The flow-cell was then washed with 100 µl of AB buffer supplemented with an oxygen scavenger enzyme system (6 mg/ml D-glucose, 40 µg/ml catalase, 200 µg/ml glucose oxidase (*96*)) and 100 mM ⍰-mercaptoethanol to reduce photobleaching. Filament movement was initiated by the infusion of 1 mM ATP, and the motility assay was carried out at 25˚C. Filament velocity was measured on digitized video sequences (usually 100 frames) by user-developed Pascal algorithms. Typical time between the digitized frames was 2 sec. Mean velocity was calculated for all filaments in a field of view. The rate of actin-filament breakage was quantitated by measuring the total number of actin filaments as a function of time immediately after the initiation of motility.