Magnetophoretic velocity of magnetic nano-objects for biomedical applications was characterized by measuring space- and time-resolved extinction profiles (STEP-Technology) using a customized LUMiReader device equipped with a set of permanent magnets (STEP-MAG). The resulting magnetic fields and gradients in a sample volume enable the operator to choose measurement conditions for magnetic micro- and nanoparticles and their assemblies. The dependence of magnetophoretic velocity on concentration and optical wavelengths indicated assembly of the nano-objects upon magnetophoresis. The method has potential applications in biomedicine to develop advanced materials and protocols for cell separation, tissue engineering, and drug/nucleic acid targeting.