Collagen/Matrigel mixed cultures for MCF-10A cells

On ice, mix:

0.5 ml Collagen Type I, 2.9-3.2 mg/ml [We get a product called Vitrogen (bovine dermal collagen) from a company called Cohesion Technologies (www.cohesiontech.com)]
62.5 ul 10x PBS (sterile filtered)
62.5 ul 0.1 M NaOH (sterile filtered)
~10 ul 0.1 N HCl to bring final pH to ~7.5

On ice, mix Collagen mix and growth factor reduced Matrigel such that the final Collagen concentration is 1.6 mg/ml (for example, if the lot of collagen is 2.9 mg/ml and you want to keep final concentration at 1.6 mg/ml, use 45% Matrigel and 55% Collagen). Spread 46 ul per well in an 8-well chamber slide. Work quickly, as the Collagen tends to solidify even on ice in a short time.

Dry in TC incubator for at least 30 minutes (I usually let it sit closer to an hour).

Add cells just as you would for a Matrigel morphogenesis overlay assay: ~5000 cells/well in 400 ul assay media with 5 ng/ml EGF and 2% Matrigel.

Feed with fresh assay media, 5 ng/ml EGF (or other required stimulus), and 2% Matrigel every four days. If adding AP1510 to activate ErbB2 homodimers, I add this instead of EGF beginning at the day 4 feeding.

I see invasive structures forming by about day 12 (8 days after inducing ErbB2 homodimers in the presence of TGF-β).