

pET24a(+)_mCerulean3

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Description

NCBI accession number: KM504958. The pOpt system was originally built with four fluorescent protein standards and one luciferase. The four fluorescent reporters, mCerulean3 (a bright cyan FP variant), mVenus (a bright yellow FP variant), Clover (a very bright green FP variant), and mRuby2 (a bright red FP variant from *Entacmaea quadricolor*), are not commercially available as protein standards. Since the Optimized expression system contains two introns (1 and 2 of RBCS2), it would require specialized reconstruction of the genes without introns for the researcher to make *Escherichia coli* expression constructs to use as standards. We, therefore, created *E. coli* codon optimized versions of the four fluorescent reporters containing the extra amino acids on C- and N-termini that are a product of the pOpt restriction sites, and included the StrepII tag on their C-terminus as is also found in the pOpt reporters. The protein products from these *E. coli* expression cassettes are identical to those produced from their pOpt cytosolic localized counterparts. The *E. coli* expression constructs are housed in the pET24a(+) backbone, driven by the T7 promoter, and are therefore inducible by addition of IPTG. pET24a(+) is Kanamycin resistant. The cell lines are *E. coli* KRX, which are designed for high recombinant protein expression purposes. These cell lines and plasmids are usable directly without any additional cloning in standard *E. coli* fermentations and StrepII affinity chromatography.

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