Prodeoxyviolacein *in vitro* assay

(optimized for our application, referring to "In Vitro Biosynthesis of Violacein from l-Tryptophan by the Enzymes VioA−E from Chromobacterium violaceum"; Carl J. Balibar and Christopher T. Walsh; Biochemistry, 2006)

The following yeast samples were harvested with 10 total OD_{600} units.

WT1	WT2	A1.1	A1.2	A2.1	A2.2	B1.1	B1.2	B2.1	B2.2	E1.1	E1.2	E2.2	E2.2

After this the purification was implemented with a protocol by invitrogen ("pGAPZ A, B, and C pGAPZα A, B, and C Pichia expression vectors for constitutive expression and purification of recombinant proteins"; invitrogen by life technologies; user manual, page 23-24, 2010).

This led to two protein- and two cell suspensions of each sample. The replicates were mixed as followed:

WT1 CS	WT2 PS	A1.1 CS	A2.1 CS	A1.2 PS	A2.2 PS	B1.1 CS	B2.1 CS	B1.2 PS	B2.2 PS	E1.1 CS	E2.1 CS	E1.2 PS	E2.2 PS
WT CS	**WT PS**	**VioA CS**		**VioA PS**		**VioB CS**		**VioB PS**		**VioE CS**		**VioE PS**	

PS = protein suspension, CS = cell suspension

mastermix (600 µl)

1200 mM glycine (54 mg)
7,8 mM FAD (38 mg)
16 units catalase per 1 µl (4,8 mg)
360 µl $MgCl_2$: 150 mM
240 µl ddH_2O

suspension mixtures

WT CS: 1500 µl WTZ
WT PS: 1500 µl WTP
CS1: 500 µl VioA CS, 500 µl VioB CS, 500 µl VioE CS
PS1: 250 µl VioA PS, 1000 µl VioB PS, 250 µl VioE PS
CS2: 500 µl VioA CS, 500 µl VioB CS, 500 µl VioE CS
PS2: 250 µl VioA PS, 1000 µl VioB PS, 250 µl VioE PS

protocol

- each reaction mix: 100 µl mastermix, 1468 µl suspension mixture
- adjust pH=9,25
- add 32 µl 1000 µM L-tryptophan to start the reaction
- incubate at 30 degrees; 400 rpm
- take 300 µl of each sample at 0, 30, 60, 90, 120 min
- add 600 µl icecold MeOH, 60 µl icecold DMSO to the sample and mix roughly
- centrifuge 5 min at 13000 rpm
- transfer supernatant in new loBind reaction tube
- incubate 10 min on ice
- centrifuge 5 min at 13000 rpm
- transfer 200 µl supernatant into LC-Vial for mass spectrometry analysis