

GEE modification of OCP under dark and light conditions

OCP Stock (OD494=120)

	Stock (M) (1ml)	Working (mM)
GEE	1.5 (0.21g/ml)	300
EDC	0.5 (0.096g/ml)	50

Setup

Treatment	OCP (ul)	Buffer (ul) Mops (10mM)	GEE stock (ul)	EDC stock (ul)	Total (ul)
Dark	5	415	120	60	600
Light	5	415	120	60	600

1. Add OCP to buffer (Mops 10 mM pH 8.0).
2. Samples to dark and light conditions (at least 10 min for fully activation, color changes within 3 min, depending on the sample concentration).
3. Add GEE first, mix, then add EDC, mix (no reverse!) and put them back to their treatment condition.
4. Allow reactions proceed (3 hours), to monitor the process, take 50 ul sample every 30 min and dilute 10x using Mops buffer to read UV-vis (300-800nm). Save all the samples.

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Setup

Treatment	OCP (ul)	Buffer (ul) Mops (10mM)	GEE stock (ul)	EDC stock (ul)	Total (ul)
Dark	10	1010	120	60	1200
Light	10	1010	120	60	1200

1. Add OCP to buffer (Mops 10 mM pH 8.0).
2. Samples to dark and light conditions (at least 10 min for fully activation, color changes within 3 min, depending on the sample concentration).
3. Add GEE first, mix, then add EDC, mix (no reverse!) and put them back to their treatment condition.
4. Allow reactions proceed (3 hours), to monitor the process, take 240 ul sample every 30 min into 12 ul (1M Tris pH 8.0), take 50 ul and dilute 10x using Mops buffer to read UV-vis (300-800nm). Save all the samples.