

***Dictyostelium* Chemotaxis to Folate: a Simple Assay from the Hadwiger Laboratory**

1. Grow cells to mid log phase $\sim 2 \times 10^6$ /ml in shaking cultures of HL-5 medium. Note: one can also use cells grown on the bottom of petri dishes but consistency of the assay might be affected by the growth status of the cells.
2. Wash cells once with phosphate buffer (12 mM NaH_2PO_4 , adjusted to pH 6.1 with KOH) and resuspend at 2×10^7 /ml. Plate 1 μl droplets of the cell suspension on nonnutrient plates (15% agar in phosphate buffer). Place 1 μl droplets of 1 mM folate (adjusted to pH 7.0 with 100 mM sodium bicarbonate) approximately 2 mm away from the cell droplet. Allow both the cell and folate droplet to absorb into the agar. Cover lid of plate and observe chemotactic movement in about 2-3 hours. Note: the droplet sizes, cell concentration, and folate concentration can be varied at least 10 fold and chemotaxis can still be observed. Folate is sensitive to light but chemotaxis will be observed whether or not the plates are maintained in the dark.
3. One should observe many cells migrating beyond the initial cell droplet perimeter (often detectable by an edge of debris formed when the droplet dries). At high cell concentrations the leading edge of chemotactic cells can contain several layers of cells - producing an incomplete halo of cells on the droplet side facing the chemoattractant source. This halo is often detectable without a microscope. Note: some cells (a much lower percentage) might also move out of the original droplet perimeter in the direction opposite of the folate source. Most of this movement is likely to be due to chemotaxis to folate that has diffused beyond the cell droplet, based on the limited amount of random cell movement observed for mutants deficient in folate reception.
4. Cells lacking the $\text{G}\alpha 4$ subunit or the $\text{G}\beta$ subunit are not responsive to folate and therefore do not chemotax in this assay. These strains are good controls for assessing cell movement that occurs in the absence of stimulation by folate.

Hadwiger, J. A., and J. Srinivasan (1999) Folic acid stimulation of the $\text{G}\alpha 4$ G protein-mediated signal transduction pathway inhibits anterior prestalk cell development in *Dictyostelium*. *Differentiation* **64**:195-204.