X-GAL STAINING OF CULTURES CELLS

Reagents:

PBS and PBS + 1mM $MgCl_2$

pH according to experiment : MEF – pH 5.5; Human – pH 6.0; LacZ – pH 7.3.

0.5% glutaraldehyde in PBS

Fresh dilution from glutaraldehyde stock at 25%

KC (20X stock)

$K_3Fe(CN)_6$	g	0,82	1,64	3,28	4,92	6,56	g
$K_4Fe(CN)_6x3H2O$	g	1,05	2,1	4,2	6,3	8,4	g
In PBS (final volume)	mL	25	50	100	150	200	mL

X-Gal (40X stock)

40 mg/mL in N,N-dimethylformamide

X-Gal staining solution (1X)

Final Volume	**PBS+$MgCl_2$**	**20X KC**	**40X X-Gal**
5	4,6	0,250	0,125
10	9,3	0,500	0,250
15	13,9	0,750	0,375
20	18,5	1,000	0,500
25	23,1	1,250	0,625
30	27,8	1,500	0,750
35	32,4	1,750	0,875
40	37,0	2,000	1,000
45	41,6	2,250	1,125
50	46,3	2,500	1,250

Tris 100mM pH 8.0

Protocol:

1. Wash cells with PBS (of appropriate pH).
2. Fix with glutaraldehyde solution for 15 minutes at room temperature.
3. Wash twice with PBS+$MgCl_2$.
4. Add X-Gal staining solution (enough to cover the plate).
5. Incubate at 37°C until coloration develops (check frequently not to over color).

   Cells can be incubated overnight at 4°C prior to incubation at 37°C to allow faster reaction the following day.
6. Wash cells with 100mM Tris pH 8.0.
7. Count and take pictures.