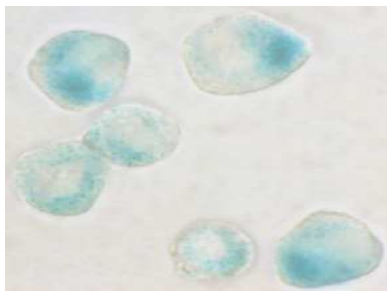


## SA- $\beta$ -Galactosidase staining

- PBS containing 1 mM MgCl<sub>2</sub>, adjust to pH 5.5 (for rodent cells; pH 6.0 for human cells)
- 50% Glutaraldehyde stock at 4°C (Roth 4995.1)
- Paraformaldehyde stock (powder at 4°C – Sigma P6148).
- **Fixative solution:** 0.25% glutaraldehyde / 2% paraformaldehyde in PBS pH 5,5 solution (1. dissolve 1g PFA in 50 ml PBS pH5,5 by heating; 2. add 250  $\mu$ l of a 50% stock glutaraldehyde solution). Store at 4°C. Do not use longer than 1-2 days
- **20x Potassium cyanide (KC) stock**
  - 820 mg K<sub>3</sub>Fe(CN)<sub>6</sub>
  - 1,050 mg K<sub>4</sub>Fe(CN)<sub>6</sub> x 3H<sub>2</sub>Oin 25 ml PBS
- **40x X-GAL solution** (i.e. 40 mg 5-bromo-4-chloro-3-indolyl  $\beta$ -D-galactoside per ml of N,N-dimethylformamide)

### Procedure for lymphoma-cells:

- Use 100.000 total cells / cytospin well.
- Cytospin cells at 700 rpm, 8 minutes.
- DO NOT DRY the slide completely
- Carefully pipet the fixative solution on the spots and incubate 10 minutes (RT)
- Wash 2 times 5 minutes in PBS/MgCl<sub>2</sub>
- Prepare **1x X-GAL solution** as follows (10ml):  
use 9.3 ml PBS/MgCl<sub>2</sub>, 0.5 ml 20x KC solution, and 0.25 ml 40x X-Gal solution
- Submerge slides in 1x X-GAL
- Incubate over night at 37°C (prevent evaporation),  
e.g. 12-16 hours (cell-dependent)
- Wash 2 times in PBS
- Mount slides while spots are still wet
- store slides at 4°C in the dark



Myc-driven B-cell-lymphoma  
expressing bcl2  
(treated with Adriamycin)  
(murine)