

## SOP A05-01: B cell depletion using Rituxan - PRELIMINARY

### Purpose and principle:

This SOP describes the method for depleting nonhuman primates of B lymphocytes using the commercial anti-CD20 antibody, Rituxan.

### Materials:

- Rituxan (rituximab, 10 mg/ml; Genentech/Biogen Idec)
- Fluorochrome-conjugated antibodies for immunophenotyping

### Procedure:

A. Schedule:	<u>Day</u>	<u>Dose</u>	<u>Route</u>
	Day 0	50 mg/kg	Intravenous
	Day 21	50 mg/kg	Intravenous
	Day 42	50 mg/kg	Intravenous

### B. Notes:

1. Pre-screening animals for pre-existing anti-Ig antibodies may be warranted but the usefulness of this has not been determined at this time.
2. The antibody needs no further dilution and can be administered as supplied.
3. The intravenous injections are given as a slow infusion over 1 to 2 hours.
4. Since this is a foreign protein, we observe monkeys carefully and are prepared for anaphylaxis, but have never observed it using this antibody.
5. At present, we recommend at least 3 treatments at the 50mg/kg dose. This treatment regimen may be continued for a longer period if needed. B cells can be expected to start returning to peripheral blood at 3 to 4 months after the last treatment.

### C. Confirmation of B lymphocyte depletion:

1. Rituxan can cross-block most anti-CD20 antibodies used for immunophenotyping. We recommend using CD19 to detect B cells.
2. Minimum recommended antibody panel for macaque monkeys:

CD	Fluorochrome	Clone	Source
CD19	PE	J4.119	Beckman Coulter
CD3	(any other color)	SP34	Pharmingen

3. We recommend CBC and immunophenotyping at weekly intervals.

#### D. References:

1. Reff ME, Carner C, Chambers KS, Chinn PC, Leonard JE, Raab R, et al. Depletion of B cells in vivo by a chimeric mouse human monoclonal antibody to CD20. *Blood* 1994, 83:435-445.
2. Schmitz JE, Kuroda MJ, Santra S, Simon MA, Lifton MA, Lin W, Khunkhun R, Piatak M, Lifson JD, Grosschupff G, Gelman RS, Racz P, Tenner-Racz K, Mansfield KA, Letvin NL, Montefiori DC, Reimann KA. Effect of humoral immune responses in controlling viremia during primary infection of rhesus monkeys with simian immunodeficiency virus. *J Virol* 2003, 77:2165-2173.

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