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Trimethoprim (TMP) injection protocol for Rasgrf2 mice

Dose: 0.25mg/g of body weight for <u>3 consecutive days.</u>

Solvent: DMSO

Delivery: Intraperitoneal injection

TMP stock solution should be prepared every day before injection.

Maximum IP injection volume allowed in mice = 10ul/g; % Weight/Volume in g/100mL.

Notes: TMP has very low solubility in aque

ous solutions so it precipitates in the needle within seconds of mixing with saline and clogs it, recommended doing in pairs. We have had high death rate in mice anaesthetised with isofluorane before injection, do in awake, restrained mice.

Protocol:

- Prepare 10% TMP in DMSO (stock solution):

| TMP (mg) | DMSO (mL) | |
|----------|-----------|--|
| 100 | 1 | |
| 500 | 5 | |
| 1000 | 10 | |

- Example mouse: 20g

 $20g \times 0.25mg/g = 5mg TMP$

 $5 \text{mg x 1g}/1000 \text{mg x } 100 \text{ml}/10 \text{g} = 0.05 \text{ml} = 50 \ \mu \text{l} \text{ of } 10\% \ \text{TMP in DMSO}$

 $20g \times 10\mu l/g = 200\mu l$ of total injected volume

 $200\mu l$ = $50\mu l$ of 10% TMP in DMSO + $150\mu l$ of Saline 0.9% NaCl

| Weight (g) | TMP (mg) | Volume (µl) 10%TMP in DMSO | Volume (µl) total DMSO + Saline |
|------------|----------|--|---|
| 15 | 3.75 | 37.5 | 150 |
| 16 | 4 | 40 | 160 |
| 17 | 4.25 | 42.5 | 170 |
| 18 | 4.5 | 45 | 180 |
| 19 | 4.75 | 47.5 | 190 |
| 20 | 5 | 50 | 200 |
| 21 | 5.25 | 52.5 | 210 |
| 22 | 5.5 | 55 | 220 |
| 23 | 5.75 | 57.5 | 230 |
| 24 | 6 | 60 | 240 |
| 25 | 6.25 | 62.5 | 250 |

| 26 | 6.5 | 65 | 260 |
|----|------|------|-----|
| 27 | 6.75 | 67.5 | 270 |
| 28 | 7 | 70 | 280 |
| 29 | 7.25 | 72.5 | 290 |
| 30 | 7.5 | 75 | 300 |

Trimethoprim: T7883-25G Sigma Aldrich; DMSO: BP231-100 Fisher