

# Two-wavelength spec

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## Instructions for the spec

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1. Turn on the spec and let it warm up for >20 minutes.
2. Mask the four edges of a cuvette with opaque tapes.
3. Make up buffer/reaction mixture (except enzyme/substrate) and place in cuvette holder.
4. Cut off tip of a p20 pipette tip and wedge this tip into the side of the cuvette to stabilize it.
5. Balance
  - a) Turn knob to  $A_{in}$  on “Uebersteuerung (Overriding)” panel. Open up A lamp all the way down and then turn “Hochspannung (High Voltage?)” knob until signal says  $\sim 0.900$
  - b) Turn knob to  $B_{in}$  on “Uebersteuerung” panel. Adjust B lamp until it matches the signal with A lamp (within 0.005)
6. Calibration
  - a) Turn on chart recorder.
  - b) Adjust gross and fine knob on the “offset” panel so that signal is  $\sim 0.000$ . Adjust zero knob on chart recorder to the leftmost line on the chart
  - c) Adjust gross and fine knob on the “offset” panel so that signal is  $\sim -1.000$ . Adjust attenuation knob on chart recorder to the rightmost line on the chart
7. Press down “chart” button on chart recorder. Start recording by pressing down “pen”. Obtain a steady baseline.
8. Open up lid on cuvette holder. In a swift motion, add enzyme/substrate onto an applicator, mix and close lid. Reaction starts.
9. When the reaction is done, press down “chart” and lift up “pen”. Turn off chart recorder and spec.

Note:

1. This machine is very sensitive. Do not touch it or lean on the bench while it's running.
2. If a red light beside the signal panel lights up during the balancing step, the voltage is too high and could shorten the life expectancy of the lamps – turn it down.
3. Filter = 334nm.
4. 1cm/min is a good chart speed.
5. Range on chart =  $1/\#$  shown on “Verstaerkung (Reinforcement/Gain)” (e.g. when gain is on 50 then the entire range on chart (from 0-1) =  $1/50$  of an OD)
6. Time length on “Filter” knob indicates that data from this period gets pooled and integrated to generate the line. ( $\therefore$  the line becomes smoother with filter is set at a longer period of time)