

**Ethanol Assay, UV-method
(R-Biopharm, Cat. No. 10 176 290 035)**

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UV cuvettes (Plastibrand Disposable UV-cuvette, micro, Cat. No. 7592 10)

Cuvette caps (Plastibrand Caps for Ultravette™, Cat. No. 759241)

~60 assays per kit

Preparation of Reagents:

All kit contents are stable at 2 to 8°C except for reaction mixture 2.

Bring solution 1 to room temperature before use.

Immediately before use, dissolve one tablet from bottle 2 in 3ml of solution 1 in a centrifuge tube for every 2 assays. This is reaction mixture 2 (stable for 1 day at 2 to 8°C, bring to room temperature before use).

Use contents of bottle 1 undiluted.

Use contents of bottle 3 undiluted.

Glucose-limited filtrates:

Use the maximum volume of sample (250µl) the assay allows for your first round of assays. If the $_A$ is above 1.00, then you must redo those samples using the smallest volume (50µl) of sample. If the $_A$ is still above 1.00 at the smallest volume, then you must dilute the sample until it is below 1.00. Change the concentration calculation accordingly.

Make sure you cap your samples at all times during the assay since ethanol is volatile.

Use the following chart to add the appropriate amount of reagents to the appropriate cuvettes:

	BLANK	SAMPLE	CONTROL
Reaction mixture 2	1500µl	1500µl	1500µl
Sample	-	50-250µl	-
Control	-	-	50µl
MilliQ	50-250µl	-	-

Cap cuvettes, and mix by inverting several times.

Read Absorbance @340nm after approx. 3 minutes (check for bubbles, gently knock out those that may interfere with the measurement)

Then add:

Suspension 3	25µl	25µl	25µl
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Cap cuvettes, and mix by inverting several times.

Read Absorbance @340nm after approx. 5 minutes.

Control Concentration (g/L) = $0.1152 \times _A$ (sample volume = 50µl)

Sample Concentration (g/L) = $0.02596 \times _A$ (sample volume = 250µl)

Important Notes

Make sure you cap your samples at all times during the assay since ethanol is volatile. According to the kit, A_{210} must be greater than or equal to 0.100 absorbance units for accurate results. You can increase your sample volume up to 250 μ l in order to achieve this minimum value. Be sure to increase the amount of MilliQ added to the blank as well.

I do not trust the spec readings if they are above 1.00. If your readings are above 1.00 at the minimum sample volume you must dilute your samples (and adjust the concentration calculation accordingly).

Mix samples well and be careful of leakage since the caps do not always form a perfect seal. Be extra careful of bubbles that may interfere with your readings. Gently knock out any bubbles before taking the absorbance measurements.

Adjust your sample values according to the difference between the actual concentration of the control solution and the calculated concentration determined using A_{210} . (For example, if your control solution is actually 0.500g/L and the calculated value is 0.492g/L, then you must multiply 0.492g/L by 1.016260163 for it to equal 0.500g/L. Thus, you must also multiply all of your sample values by the same number.)