

Gain Settings

GAIN

The LED excitation sources used by the Thermo Scientific NanoDrop™ 3300 Fluorospectrometer are activated for a sample specific period of time to maximize their respective emission. We use the term “gain” to refer to the amount of time the sample is excited. Time is, therefore, used to maximize the signal to the CCD detector while preventing detector saturation.

AUTO/FIXED GAIN

Auto/Fixed Gain are the two options for setting the gain or excitation time and are selected via the Create/Edit method module.

- When the Auto Gain (default) mode is used, the source (LED) illumination (excitation) time is optimized for each sample. The Actual Gain value for the respective sample will be displayed in this mode. A value of 10.0 corresponds to the maximum gain or excitation time maximum of 1 second (1000 msec).
- The Fixed Gain option is selected when constant gain or excitation time is wanted for every sample. Fixed Gain settings range from 0.1 to 10.0 in 0.1 increments.

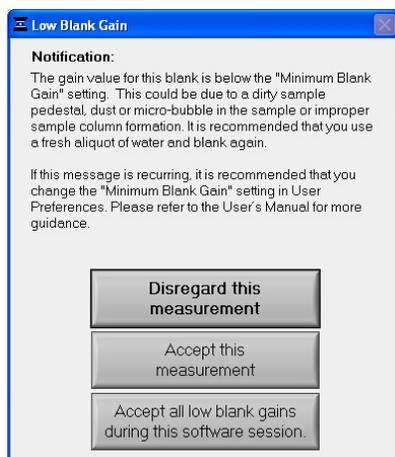
The measurement of fluorescence is always reported in RFU or relative (non-absolute) fluorescent units. RFU signals for all gain settings are normalized for time for each measurement.

MIN BLANK GAIN (Blank Measurements)

This parameter is individually set for each LED and determines the minimum excitation time used for making reference or “blank” measurements. The default setting of “5” is usually the most appropriate for both the Blue and White LED. The default setting of “10” is the most appropriate for the UV LED.

LOW BLANK GAIN NOTIFICATION

This message is automatically displayed when the actual gain for a blank is less than that specified as the default setting. It is recommended that the user blot away the water or buffer, clean the pedestal area of dust and lint load another sample and make a new blank measurement. If the warning re-occurs for three successive measurements, it is recommended that the user accept the measurement and reset the default Min Gain settings for the Blue or White LEDs as appropriate.



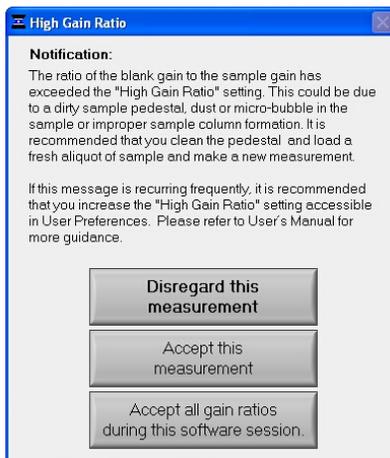
We recommend reviewing the blank gain settings in the archived data and choosing a gain value consistent with the recorded data. Gains of less than 1 are not acceptable for Min Blank Gain settings.

MIN GAIN RATIO (Sample Measurements)

This factor compares the actual gain used for the sample measurement to the actual gain used for the blank measurement. If the ratio exceeds the Max Gain ratio setting, a message will appear prompting the user to either accept or reject the measurement. If the spectral image is particularly distorted or has very narrow emission profiles, it is best to reject the measurement and re-measure with a fresh aliquot. The signal (RFU) is always displayed and can be used to assess the validity of the message. The default setting for the minimum gain ratio is 3.

HIGH GAIN RATIO NOTIFICATION

This message is automatically displayed when ratio of the actual sample gain to the blank gain is higher than that specified as the default setting. It is recommended that the user blot away the water or buffer, clean the pedestal area of dust and lint, load another sample and make a new measurement.



SATURATION nm

The operating software automatically limits the amount of time the LED source is on to prevent too much light from saturating the detector for all wavelengths above the “saturation nm”. The default setting is 25nm below the selected emission wavelength for the Blue and UV LEDs and 40 nm below the emission wavelength for the White LED. The user may find that for a particular application, choosing a saturation nm nearer the emission nm of interest may result in longer excitation times and higher sample fluorescence

For technical support contact us at 302 479-7707 or info@nanodrop.com.

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