For use in quality control/
manufacturing process only.

Density Reference Standard Beads (DRSB)

Version 53

Content version: May 2021

Beads for one-point density calibration

Cat. No. 06 422 659 001 Batch A

Store Beads at +2 to +8°C

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1. Introduction

The Cedex HiRes Analyzer measures the cell density, also known as concentration, of a cellular suspension, along with its viability status and cell characteristics such as diameter and compactness. In order to check the correct calibration with regard to the density, use the Density Reference Standard Beads instead of an ordinary cell sample.

This product is traceable in the following aspects:

- Particle Size: NIST, USA
- Particle Concentration: Physikalisch-Technische Bundesanstalt, Berlin, Germany

The Density Reference Standard Beads are made to mimic cell behavior in flow dynamics. Due to their size and optical properties, they will appear as dead cells in the Cedex Software.

Contents

Content	Volume	Cat. No.
Beads for one-point density calibration	10 ml	06 422 659 001

Storage and Stability

Store Beads at +2 to +8°C.

The product is stable until the expiry date printed on the label, when handled as described in these Instructions for Use.

2. How to Use this Product

There is no general advice with regard to how often or how many counts should be done in order to ensure that your analyzer is working correctly. Roche Diagnostics has had good results using the DRSB on a monthly basis, carrying out 10 samples per run with the Cedex HiRes Analyzer.

Two factors influence the acceptance range for calibration with Density Reference Standard Beads in connection with the Cedex HiRes Analyzer.

- **Sample preparation:** Mixing, pipette operation, and pipette quality (precision, accuracy, service state) have been shown in the field to add approximately 1.5 – 2% to the variability in density measurements.
- **Measurement precision is based on the statistical nature of the measurement process.** It depends on the density of the DRSB used, Cell Type parameter settings, and the level of precision used for the measurement.

Sampling quality is essential for the evaluation of the status of the instrument. Special care should be taken to ascertain, for example, that among other factors:

- Beads were not frozen, but stored properly at +2 to +8°C.
- Beads were allowed to acclimate to a temperature of +23 to +27°C prior to use.
- Weight of the unopened bottle is correct (see label on the bottle).
- Ultrasonic bath is used for mixing.
- Bottle is rocked gently, including rocking upside down.
- No more than 2 samples are drawn from the bottle without intermediate remixing.
- Only calibrated pipettes are used.
- Only trained staff are performing the sample preparation.

3. Protocol

3.1 Preparation of the DRSB solution

- Verify that the beads have been stored correctly at temperatures of +2 to +8°C (BEADS CANNOT BE FROZEN).
- Verify that the bottle was securely closed before use (check the weight of the unopened bottle; the correct value is given on the bottle).
- Allow the beads to acclimate to a temperature of +23 to +27°C prior to use.
- Use an ultrasonic bath at a temperature of +23 to +27°C and at the highest available intensity for 5 minutes (with cap slightly loosened but secured against falling over) to shake the beads.
- Ensure that no beads are sticking to the base or side of the bottle before use.

The DRSB solution contains SDS, which may show signs of some coagulation or crystallization at low temperatures. Crystals and/or signs of coagulation can be removed by allowing the beads to acclimate, with occasional mixing, at +25°C until the coagulation disappears. Alternatively, the DRSB bottle can be gently rolled between the palms of the hands until the coagulation has disappeared. Note that as long as the DRSB solution has been allowed to acclimate to +23 to +27°C, and all steps in this Instructions for Use have been carried out, any remaining coagulation or crystallization will have no effect on the performance or quality of the DRSB solution when used in a Cedex HiRes Analyzer.

3.2 Checking the FlowFactor (FF) (see Figure 2)

❶	Pipet 1 sample of 0.3 ml DRSB into a Cedex Sample cup, and run the sample with factory settings for default Cell Type Std. Size immediately. Select the maximum possible setting for “precision”.
❷	Mix the DRSB thoroughly, then pipet the next sample of 0.3 ml into a Cedex Sample cup, and run the sample immediately.
❸	Repeat this procedure until 10 samples are processed.
❹	Calculate the mean value of the Total Cell Density (TCD) of the 10 samples used.
❺	Calculate the relative standard deviation of the TCD values of the 10 samples used, and verify that the relative standard deviation is less than or equal to 5%. Otherwise, the Cedex HiRes Analyzer, the beads, or the handling have to be checked and the calibration must be repeated.
❻	Calculate the deviation of the mean TCD value of the 10 samples used from the actual value (given as Particle number/ml on the bottle of beads).
❼	Verify that the deviation of the mean TCD value is less than or equal to 5%, or as specified by your requirements, from the actual value given on the bottle for the beads. If the value falls outside of the acceptable range, skip to Step 9.
❽	Close bottle tightly and store beads at +2 to +8°C (BEADS CANNOT BE FROZEN). The current FF is correct and no change is necessary.
❾	If the mean value falls out of range, repeat Steps 1 to 5 using a second/different LOT (batch) of Density Reference Standard Beads. Continue with Step 10.
❿	Calculate the FlowFactor (FF) of each measurement series (see 5.1, “How to Calculate and Change the FlowFactor”), and the mean value of the two FFs.
⓫	Verify that the deviation of the two FFs from the mean value of the FFs, are less than or equal to 5%. Otherwise, the Cedex HiRes Analyzer, the beads, or the handling have to be checked and the calibration must be repeated.
⓬	Calculate the new FF (mean value of the FFs); (see 5.1, “How to Calculate and Change the FlowFactor”), or follow your company’s requirements.

4. Lot Specific Data

Cat. No. 06 422 659 001, Batch A

Valid for Lot. No. 57130059

In this chapter, you will find lot specific data about your product. The table below provides the following information for each bottle produced for this lot.

Column 1: Bottle No. for the bottle.
Column 2: Actual concentration expressed in particle number/ml for the bottle.
Column 3: Total weight of bottle, including bottle, contents, and label.
Column 4: Check Box for marking which bottle was received.

Use this table as follows:

❶	Print out the table.
❷	Find the bottle number on the bottle label as shown in Figure 1.
❸	Place a check mark in the "Bottle Received" column to mark the specific bottle received for future reference.

This product is traceable in the following aspects:

- Particle Size: NIST, USA
- Particle Concentration: Physikalisch-Technische Bundesanstalt, Berlin, Germany

Fig. 1: Example of how to find the bottle number on the bottle label. The bottle number is circled.

ID-Nr. LOT 57130059	Concentration Particle number/ml (10^5)	weight (g)	Bottle received
A1	10.13	29.389	
A2	10.04	29.344	
A3	10.06	29.355	
A4	10.07	29.346	
A5	10.10	29.315	
A6	10.11	29.440	
A7	9.97	29.362	
A8	10.08	29.386	
A9	10.08	29.283	
A10	10.14	29.365	
A11	10.03	29.502	
A12	10.01	29.283	
A13	10.13	29.407	
A14	10.06	29.626	
A15	10.00	29.519	
A16	10.12	29.550	
A17	10.09	29.231	
A18	10.07	29.350	
A19	10.05	29.395	
A20	10.05	29.472	
A21	10.14	29.267	
A22	10.10	29.307	
A23	10.02	29.335	
A24	10.06	29.361	
A25	9.99	29.394	
A26	9.97	29.241	
A27	10.06	29.357	
A28	10.11	29.420	
A29	10.00	29.411	

ID-Nr. LOT 57130059	Concentration Particle number/ml (10^5)	weight (g)	Bottle received
A30	10.00	29.630	
A31	10.06	29.349	
A32	9.98	29.391	
A33	10.09	29.498	
A34	10.10	29.484	
A35	10.05	29.441	
A36	10.16	29.398	
A37	10.06	29.349	
A38	10.12	29.380	
A39	10.08	29.351	
A40	10.12	29.422	
A41	10.06	29.162	
A42	10.08	29.402	
A43	10.07	29.618	
A44	10.07	29.322	
A45	10.00	29.539	
A46	10.09	29.526	
A47	10.06	29.236	
A48	10.10	29.363	
A49	10.10	29.489	
A50	10.19	29.538	
A51	10.01	29.278	
A52	10.01	29.403	
A53	10.14	29.643	
A54	10.04	29.343	
A55	9.98	29.309	
A56	10.04	29.329	
A57	9.99	29.486	
A58	10.02	29.348	

ID-Nr. LOT 57130059	Concentration Particle number/ml (10^5)	weight (g)	Bottle received
A59	10.00	29.428	
A60	9.98	29.485	
A61	10.16	29.417	
A62	10.18	29.534	
A63	10.15	29.493	
A64	10.13	29.264	
A65	10.05	29.506	
A66	10.06	29.259	
A67	9.95	29.467	
A68	10.06	29.471	
A69	10.00	29.419	
A70	10.10	29.495	
A71	10.14	29.231	
A72	10.06	29.444	
A73	10.08	29.514	
A74	10.02	29.536	
A75	10.01	29.604	
A76	10.17	29.418	
A77	10.06	29.437	
A78	10.15	29.646	
A79	10.17	29.524	
A80	10.07	29.382	
A81	10.14	29.388	
A82	10.15	29.411	
A83	10.12	29.489	
A84	10.03	29.376	
A85	10.08	29.438	
A86	10.03	29.403	
A87	10.04	29.404	

ID-Nr. LOT 57130059	Concentration Particle number/ml (10^5)	weight (g)	Bottle received
A88	10.03	29.358	
A89	10.08	29.277	
A90	10.03	29.516	
A91	10.02	29.210	
A92	10.01	29.358	
A93	10.08	29.503	
A94	9.96	29.460	
A95	9.99	29.421	
A96	10.16	29.350	
A97	9.99	29.532	
A98	10.20	29.392	
A99	9.98	29.637	
A100	10.22	29.476	
A101	10.08	29.626	
A102	10.12	29.406	
A103	10.04	29.687	
A104	10.00	29.568	
A105	10.05	29.520	
A106	10.08	29.622	
A107	10.02	29.753	
A108	10.02	29.500	
A109	9.98	29.497	
A110	9.95	29.641	
A111	10.12	29.624	
A112	10.07	29.656	
A113	10.23	29.489	
A114	10.23	29.534	
A115	10.09	29.490	
A116	10.11	29.753	

ID-Nr. LOT 57130059	Concentration Particle number/ml (10^5)	weight (g)	Bottle received
A117	10.01	29.705	
A118	10.08	29.452	
A119	10.09	29.532	
A120	10.07	29.599	
A121	10.02	29.665	
A122	10.09	29.499	
A123	10.10	29.553	
A124	10.08	29.613	
A125	10.12	29.581	
A126	10.10	29.490	
A127	10.03	29.795	
A128	10.03	29.472	
A129	9.96	29.429	
A130	10.07	29.732	
A131	10.11	29.730	
A132	10.15	29.704	
A133	10.07	29.513	
A134	10.09	29.505	
A135	10.10	29.788	
A136	10.11	29.505	
A137	10.09	29.485	
A138	10.02	29.604	
A139	10.03	29.436	
A140	10.06	29.780	
A141	10.11	29.620	
A142	10.10	29.680	
A143	10.13	29.439	
A144	10.03	29.748	
A145	10.15	29.569	

ID-Nr. LOT 57130059	Concentration Particle number/ml (10^5)	weight (g)	Bottle received
A146	10.17	29.528	
A147	10.06	29.623	
A148	10.06	29.556	
A149	10.09	29.309	
A150	10.07	29.447	
A151	10.09	29.846	
A152	10.11	29.921	
A153	10.18	29.707	
A154	10.13	29.605	
A155	10.01	29.625	
A156	10.00	29.646	
A157	10.11	29.534	
A158	10.06	29.599	
A159	10.05	29.555	
A160	10.04	29.838	
A161	10.19	29.939	
A162	10.16	29.614	
A163	10.01	29.740	
A164	10.25	29.712	
A165	10.12	29.832	
A166	10.14	29.589	
A167	10.11	29.559	
A168	10.12	29.682	
A169	10.18	29.476	
A170	10.13	29.676	
A171	10.14	29.546	
A172	10.15	29.660	
A173	10.18	29.646	
A174	10.00	29.441	

ID-Nr. LOT 57130059	Concentration Particle number/ml (10^5)	weight (g)	Bottle received
A175	10.04	29.396	
A176	9.98	29.450	
A177	10.03	29.444	
A178	10.02	29.487	
A179	10.14	29.498	
A180	10.12	29.518	
A181	10.12	29.614	
A182	10.16	29.645	
A183	10.06	29.488	
A184	10.14	29.427	
A185	10.09	29.729	
A186	10.11	29.593	
A187	10.01	29.626	
A188	10.04	29.697	
A189	10.12	29.532	
A190	10.03	29.800	
A191	10.09	29.305	
A192	10.10	29.486	
A193	10.04	29.396	
A194	9.96	29.636	
A195	10.01	29.481	
A196	10.14	29.566	
A197	10.09	29.444	
A198	10.09	29.639	
A199	10.15	29.533	
A200	10.12	29.751	
A201	10.07	29.726	
A202	9.96	29.412	
A203	10.02	29.586	

ID-Nr. LOT 57130059	Concentration Particle number/ml (10^5)	weight (g)	Bottle received
A204	10.01	29.508	
A205	9.96	29.785	
A206	10.02	29.868	
A207	10.07	29.747	
A208	10.02	29.632	
A209	9.98	29.574	
A210	10.15	29.646	
A211	10.16	29.394	
A212	10.10	29.794	
A213	10.13	29.715	
A214	10.15	29.412	
A215	10.08	29.516	
A216	10.20	29.657	
A217	10.09	29.622	
A218	10.03	29.493	
A219	10.00	29.681	
A220	10.17	29.719	
A221	10.06	29.308	
A222	10.10	29.697	
A223	10.11	29.658	
A224	10.05	29.642	
A225	10.11	29.656	
A226	10.11	29.670	
A227	10.00	29.500	
A228	10.03	29.497	
A229	10.06	29.314	
A230	10.06	29.612	
A231	10.07	29.684	
A232	10.14	29.602	

ID-Nr. LOT 57130059	Concentration Particle number/ml (10^5)	weight (g)	Bottle received
A233	10.12	29.472	
A234	10.06	29.463	
A235	10.02	29.352	
A236	10.15	29.698	
A237	10.19	29.693	
A238	10.23	29.703	
A239	10.15	29.616	
A240	10.23	29.663	
A241	10.11	29.505	
A242	10.10	29.594	
A243	10.12	29.638	
A244	10.12	29.649	
A245	10.04	29.466	
A246	10.09	29.684	
A247	10.17	29.570	
A248	10.13	29.708	
A249	10.04	29.422	
A250	10.04	29.712	
A251	10.02	29.567	
A252	10.10	29.501	
A253	10.14	29.746	
A254	10.13	29.561	
A255	10.19	29.375	
A256	10.12	29.870	
A257	10.17	29.998	
A258	10.07	30.304	
A259	10.16	29.480	
A260	10.06	29.732	
A261	10.19	29.766	

ID-Nr. LOT 57130059	Concentration Particle number/ml (10^5)	weight (g)	Bottle received
A262	10.02	29.395	
A263	10.19	29.638	
A264	10.12	29.649	
A265	10.11	29.743	
A266	10.05	30.124	
A267	10.06	29.944	
A268	10.19	29.492	
A269	10.04	29.665	
A270	10.16	29.714	
A271	10.07	29.239	
A272	10.09	29.531	
A273	10.10	30.013	
A274	10.20	30.262	
A275	10.18	30.388	
A276	10.18	29.510	
A277	10.05	29.543	
A278	10.12	29.626	
A279	10.11	29.487	
A280	10.04	29.342	
A281	10.08	29.630	
A282	10.07	29.592	
A283	10.20	29.411	
A284	10.10	29.698	
A285	10.02	29.667	
A286	10.02	29.573	
A287	10.04	29.559	
A288	10.11	29.630	
A289	10.02	29.556	
A290	10.16	29.698	

ID-Nr. LOT 57130059	Concentration Particle number/ml (10^5)	weight (g)	Bottle received
A291	10.16	29.660	
A292	10.19	29.584	
A293	10.22	29.741	
A294	10.12	29.398	
A295	10.18	29.561	
A296	10.13	29.734	
A297	10.20	29.629	
A298	10.02	29.337	
A299	10.03	29.431	
A300	10.08	29.618	
A301	10.17	29.785	
A302	9.97	29.659	
A303	10.07	29.561	
A304	10.08	29.563	
A305	10.03	29.697	
A306	10.07	29.727	
A307	10.06	29.537	
A308	10.06	29.604	
A309	10.03	29.638	
A310	10.17	29.516	
A311	10.07	29.715	
A312	10.06	29.554	
A313	10.04	29.586	
A314	10.05	29.618	
A315	10.24	29.506	
A316	10.07	29.660	
A317	9.96	29.638	
A318	10.07	29.697	
A319	9.98	29.594	

ID-Nr. LOT 57130059	Concentration Particle number/ml (10^5)	weight (g)	Bottle received
A320	10.01	29.719	
A321	10.08	29.444	
A322	10.23	29.632	
A323	10.13	29.664	
A324	9.96	29.651	
A325	10.17	29.627	
A326	10.04	29.563	
A327	10.17	29.802	
A328	10.04	29.634	
A329	10.02	29.564	
A330	10.08	29.564	
A331	10.12	29.680	
A332	10.23	29.443	
A333	10.09	29.709	
A334	10.13	29.636	
A335	10.06	29.777	
A336	10.02	29.427	
A337	10.18	29.614	
A338	10.01	29.665	
A339	10.12	29.610	
A340	9.98	29.722	
A341	9.99	29.400	
A342	10.00	29.696	
A343	9.96	29.643	
A344	10.05	29.617	
A345	10.00	29.627	
A346	10.16	29.659	
A347	10.14	29.625	
A348	10.04	29.683	

ID-Nr. LOT 57130059	Concentration Particle number/ml (10^5)	weight (g)	Bottle received
A349	10.03	29.583	
A350	10.16	29.700	
A351	10.07	29.557	
A352	10.18	29.708	
A353	10.09	29.669	
A354	10.02	29.738	
A355	10.10	29.563	
A356	10.06	29.650	
A357	10.16	29.597	
A358	10.00	29.734	
A359	10.07	29.664	
A360	10.23	29.699	
A361	10.13	29.741	
A362	10.00	29.709	
A363	10.11	29.793	
A364	10.06	29.639	
A365	10.02	29.663	
A366	10.12	29.627	
A367	10.06	29.739	
A368	10.03	29.695	
A369	10.03	29.601	
A370	10.23	29.718	
A371	10.10	29.474	
A372	10.16	29.595	
A373	10.19	29.624	
A374	9.96	29.981	
A375	10.21	29.764	
A376	10.08	29.782	
A377	10.05	29.724	

ID-Nr. LOT 57130059	Concentration Particle number/ml (10^5)	weight (g)	Bottle received
A378	10.06	29.749	
A379	10.07	29.749	
A380	10.04	29.747	
A381	10.05	29.921	
A382	10.05	30.058	
A383	10.16	30.011	
A384	10.10	29.999	
A385	10.04	30.000	
A386	10.17	29.875	
A387	10.08	29.750	
A388	10.16	29.816	
A389	10.11	29.647	
A390	10.09	29.887	
A391	10.02	29.461	
A392	10.03	29.645	
A393	10.00	29.777	
A394	10.09	29.562	
A395	10.00	29.613	
A396	10.10	29.524	
A397	10.06	29.651	
A398	10.04	29.508	
A399	10.04	29.638	
A400	9.95	29.634	
A401	10.13	29.812	
A402	10.09	29.290	
A403	10.14	29.566	

5. Adjusting the FlowFactor

The FlowFactor (FF) is analyzer specific and part of the conversion factor that relates the number of objects detected in the Cedex HiRes Analyzer to the actual density in the analyzed sample.

The conversion factor is linearly dependent on the FlowFactor, thus allowing for the possibility to compute the appropriate setting for this parameter via the comparison of Cedex HiRes Analyzer results versus a known density of a sample (*e.g.*, Density Reference Standard Beads).

Refer to the relevant Cedex HiRes Operator's Manual for the location of the current FlowFactor. The location depends on the installed Software version.

5.1 How to Calculate and Change the FlowFactor

❶ Write down the current FlowFactor (FFold) and calculate a new FlowFactor as follows:

$$FF(new_1) = \frac{\text{actual density (according to bottle label)}}{\text{mean value TCD of measurement series 1}} \times FF(old)$$

$$FF(new_2) = \frac{\text{actual density (according to bottle label)}}{\text{mean value TCD of measurement series 2}} \times FF(old)$$

("actual density" is specified as Particle number/ml on the label of the bottle of beads used for the calibration.)

$$\textbf{FF (new)} = \frac{FF(new_1) + FF(new_2)}{2}$$

❷ Refer to the relevant Cedex HiRes Operator's Manual for information about the location of the FlowFactor. Update the FlowFactor in that location based on the result calculated in Step 1.

5.2 FlowFactor Calibration

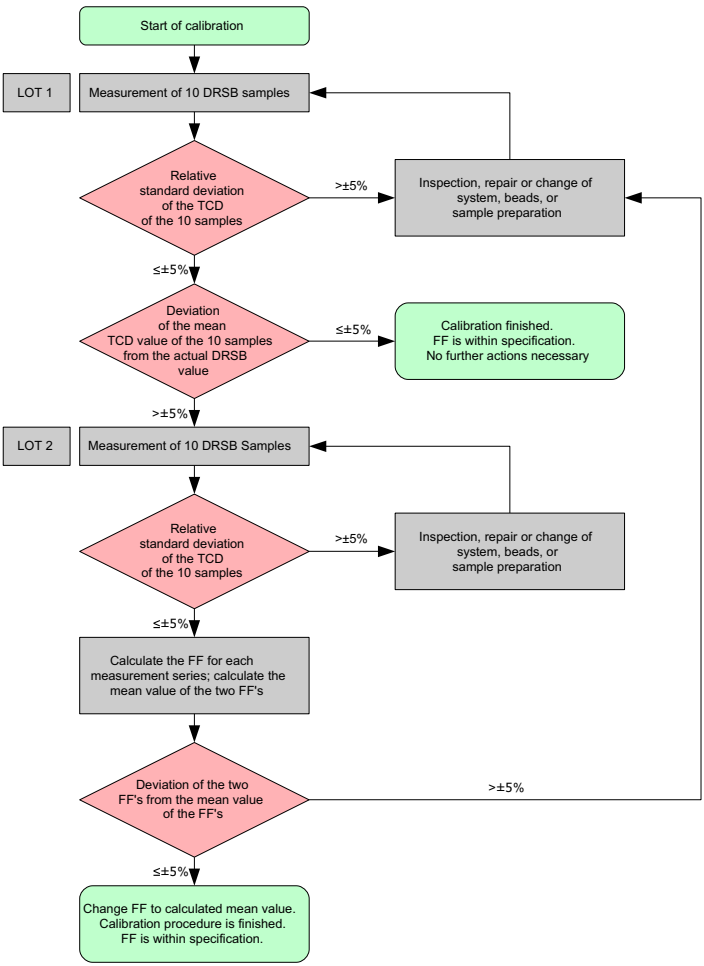

Fig. 2: Calibration Cedex HiRes Analyzer

6. Supplementary Information

6.1 Conventions

6.1.1 Text Conventions

To make information consistent and easier to read, the following text conventions are used in this document:

Text Convention	Usage
Numbered stages labeled ①, ②, etc.	Stages in a process that usually occur in the order listed.
Numbered instructions labeled ❶, ❷, etc.	Steps in a procedure that must be performed in the order listed.
Asterisk *	Denotes a product available from Roche Diagnostics.

6.1.2 Symbols

In this document, the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
⚠	Important Note: Information critical to the success of the procedure or use of the product.

6.2 Changes to Previous Version

- Updated to include lot-specific data for new lot.
- Updated of the contact and support link and the US customer service phone.

6.3 Trademarks

CEDEX is a trademark of Roche.
Other brands or product names are trademarks of their respective holders.

6.4 Regulatory Disclaimer

For use in quality control/manufacturing process only.

6.5 Contact Support

For additional documentation such as certificates and safety data sheets, please visit documentation.roche.com.

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For more information about this product please visit documentation.roche.com
For more documentation such as instructions for use and safety data sheets, please visit documentation.roche.com

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